Dissertation

"PROBIOTICS IN DIABETIC WOUND CARE"

Submitted in Partial Fulfillment of

Requirements for

M.S. BRANCH - I

GENERAL SURGERY

MADRAS MEDICAL COLLEGE

THE TAMILNADU

Dr. MGR MEDICAL UNIVERSITY

CHENNAI – TAMILNADU

APRIL 2017

CERTIFICATE

This is to certify that, the dissertation entitled **"PROBIOTICS IN DIABETIC WOUND CARE"** is the bonafide work done by **Dr. CHOUNDAPPAN M.** during his MS (General Surgery) course 2014- 2017, done under my supervision and is submitted in partial fulfilmentfor the requirement of the M.S. (BRANCH-I) General Surgery, April 2017 examination of The Tamilnadu Dr. MGR Medical University.

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DECLARATION

I, Dr.Choundappan M declare that this dissertation titled **"PROBIOTICS IN DIABETIC WOUND CARE"** represents a genuine work of mine. The contributions of any supervisors to the research are consistent with normal supervisory practice, and are acknowledged.

I also affirm that this bonafide work or part of this work was not submitted by me or any others for any award, degree or diploma to any other University board, either in India or abroad. This is submitted to The Tamilnadu Dr. M.G.R Medical University, Chennai in partial fulfilment of the rules and regulations for the award of Master of Surgery Degree Branch I (General Surgery).

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CERTIFICATE OF APPROVAL

To
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Dear Dr.M.Choundappan,

The Institutional Ethics Committee has considered your request and approved your study titled **"PROBIOTICS IN DIABETIC WOUND CARE " NO.27012016.**

The following members of Ethics Committee were present in the meeting held on **12.01.2016** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
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We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

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M.S. BRANCH - I
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Acknowledgement

The success and final outcome of this project required a lot of guidance and assistance from many people and I am extremely fortunate to have got this all along the completion of my project work. Whatever I have done is only due to such guidance and assistance and I would not forget to thank them.

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Abstract

Background

Diabetic foot is a leading cause of DALY with both physical and economic cost to the patient. It can range from a small trophic ulcer to frank sepsis and shock. With increasing cost of healthcare and antibiotic resistance, there is a need for new and innovative methods for management of the diabetic foot ulcers.

Aims and objectives

To study the effect of local application of probiotics on the healing of Diabetic foot ulcers

1. To compare the change in wound bed score in the test and control population
2. To compare the wound swab culture results in the test and control population

Methodology

Diabetic patients presenting with acute infected ulcers of the foot (below ankle) are taken up for surgical debridement on the day of presentation. The size of their wounds are assessed by wound tracing and planimetry method. The patients are screened for peripheral vascular disease by using ankle brachial pressure index. The patients are also screened for peripheral neuropathy. The patients who consented to participate in the study were allocated into two. The

control group where the current regimen of sharp and chemical debridement at ward, cleaning and dressing, glycemic management and antibiotic therapy is given. In the intervention group, in addition to the above, probiotic solution is applied daily during dressing. Wound bed scoring system developed by Falanga was utilised to monitor the wound in an objective manner. Wound swab cultures are taken at Day 0, Day 5 and Day 10. Both the groups will be compared with respect to the wound bed score at day 1, day 7 and day 14 and the wound swab cultures and outcomes identified. The results were analysed.

Results

1) A total study population of 36 was analysed
2) All the patients had improvement in the wound status
3) The mean wound bed score of the intervention group was better than the control group
4) The wound swab culture report was also reported as no growth in more number in the intervention group however it was not statistically significant.

Conclusion

1. Probiotics can be safely utilized in therapy of infected diabetic wounds
2. They do hasten the wound healing process as evidenced by the significant difference in the day 7 wound bed score
3. More studies are needed in this field to give better evidence for the support of probiotic use

Keywords

Probiotics, diabetic foot ulcer, lactobacillus, gangrene.

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Introduction

The diabetic foot is an important cause of mortality and morbidity. It ranges from an uninfected chronic ulcer to frank gangrene of the limb. It causes great physical handicap, and psychosocial disability. Also the economic cost with respect to health care expenditure, loss of work days, indirect costs to the patient are high. Moreover, with due to the indiscriminate use of antibiotics the problem of antibiotic resistance is a rapidly rising one. So novel therapies and interventions are the need of the hour to both reduce the cost, time and deal with the antibiotic resistance problem. There were only 36 papers dealing specifically with diabetic foot in the Pubmed database for the past 5 years, as compared to other complications of diabetes like nephropathy which has 8000 papers. One of the novel technology is the application of probiotics. The research in probiotics is at a nascent stage. Few studies in mice, rats and even one study on burns patients have given positive evidence regarding the probiotics. Hence, further research in this field is need of the hour.

Aims and objectives

To study the effect of local application of probiotics on the healing of Diabetic foot ulcers

1. To compare the change in wound bed score in the test and control population
2. To compare the wound swab culture results in the test and control population

Review of literature

Diabetes

Diabetes mellitus refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Genetics and environmental factors by complex interaction cause several distinct types of diabetes mellitus. Depending on the etiology of the diabetes mellitus, factors such as reduced insulin secretion, decreased glucose utilization and increased glucose production contribute to hyperglycemia. Pathophysiologic changes in multiple organ systems impose a tremendous burden on diabetic individuals and health care systems. End stage renal disease is an important complication of diabetes mellitus. End stage renal disease increase the incidence of cardiovascular complications. Lower extremity amputations for non-traumatic reasons are increased by diabetes mellitus. Adult blindness is also predisposed by Diabetes Mellitus. Diabetes mellitus is a leading cause of mortality and morbidity.

Diabetes and gangrene

Marchal de Calvi in 1852 first recognized the association between gangrene and diabetes (Marchal de Calvi A. Des rapports de la gangr`ene et de la glycosurie. *Gazette des H`opitaux Civils et Militaires* 1852; **25**:178.). Also, in 1864 he was one of the earliest to recognize an interconnection between diabetes and peripheral nerve damage and described dorsal ulcers on clawed toes. Thomas Hodgkin in 1854 made further studies in this field. Another Frenchman, M Laffon in 1885 probably first identified the association between diabetes and

plantar neuropathic ulceration (Anon. Annotation: perforating ulcer in diabetes. *Lancet* 1885; **2**588.). Complications of diseases of peripheral nerves and spinal cord are recognized as perforating or tophic ulcers i.e. Neuropathic foot ulcers. It was recognized that the persistence and pathogenesis of such ulcers were dependent on the presence of pressure and callus. It was realized that prolonged bed rest could heal such ulcers, but they promptly recurred once the patient was mobilized. Persistence and recurrence of ulcers were often indications for amputation of part of the foot. However, ulceration in the amputation stump or deformed foot was an expected complication. Novel approaches were developed. For example, an artificial limb was articulated to the flexed knee thereby preventing the foot from weight bearing. This treatment was suggested by Savory and Butlin. Frederick Treves described in 1884 a method of applying linseed poultices to soften the callus. This could be shaved away with a scalpel and the process repeated as often as necessary until the ulcer was surrounded by thin fresh pink epidermis, looking active and healthy (Treves F. Treatment of perforating ulcer of the foot. *Lancet* 1884; **2**: 949–951). This he realized greatly shortened the period of bed rest required to heal such ulcers. This also was probably the first description of the use of sharp debridement for the treatment of trophic ulcers. He further described the treatment of such ulcers which included discontinuation of such poultices, and treatment of the ulcer crater with a cream composed of a little carbolic acid, salicylic acid and glycerine which would have probably had some antiseptic properties. The necessity for a reduction in pressure to prevent recurrence was recognized by Treves. He

suggested his patients to wear a thick pad of felt plaster over the spot with a hole in the centre that corresponds to the scar of the recent sore. He felt that this plaster should be compulsorily worn. He realized that further ulceration at other pressure points could not be prevented by this method. However this complication had not yet happened in his patients. Patients were instructed by Treves to pay great attention to the cleanliness of the feet, to wear well-fitting woollen stockings and easy boots. By 1884, the three guiding principles in the treatment of neuropathic ulceration were established by Treves. They included education about foot care and footwear, offloading of pressure for both treatment and prevention, sharp debridement. For the next 40 years however, these three principles were largely forgotten.

In 1893, the British surgeon Godlee, first recognized the distinction between gangrene due to vascular insufficiency and infective gangrene in a limb with near normal blood supply. This was important because the prognosis in cases of gangrene associated with vascular disease was much worse than those cases associated with neuropathy and infection. A minor amputation or local excision of the lesion must heal successfully in which vascular disease was not a contributing factor, but those lesions caused by diseases of the vascular system usually required a major amputation. Even minor surgery could precipitate hyperglycaemic coma and death in earlier days when insulin was yet to be discovered. Hence surgery was avoided and even gangrene would often be conservatively managed. However such treatment courses succeeded only in small number of cases as in a well demarcated gangrene affecting only a toe.

Often major surgery was the only course of treatment which could offer any benefit in those patients with more extensive or rapidly spreading gangrene. A high incidence of sloughing and infection was common in these amputation flaps. All these contributed to a general reluctance to operate. Only after aseptic surgery was introduced by Godlee and others in America and Britain, the incidence of stump infection and necrosis was no longer as high as it had been. Gangrene had been second only to coma as a cause of death in patients with diabetes in the preinsulin era. After the introduction of insulin, the proportion of deaths due to foot disease increased while those due to coma decreased. Introduction of penicillin was a major advancement in the medical management of diabetic foot disease. For thirty years after it was introduced no new major advancements were made. A PubMed search showed that in each decade from 1950 onwards the number of papers published on the diabetic foot was lesser than those on retinopathy, nephropathy or neuropathy. This trend continued till the end of 1980s. Only in the 1990s the trend started to reverse, however even now the number of research papers on foot disease is less than the numbers for any other complications as shown in the table below:

Table 1: Distribution of research papers indexed on PubMed on various complications of diabetes						
Diabetic	1960-1969	1970-1979	1980-1989	1990-1999	2000-2010	2010-2016
Diabetic foot	16	35	54	114	123	36
Neuropathy	149	373	1603	3041	4728	4493
Nephropathy	693	1347	3014	7004	12843	8115
Retinopathy	1114	2178	3563	5066	8199	7106

Neuropathy

Degenerative changes of axons are the hallmark of diabetic peripheral neuropathy. It affects all nerve fibres at varying points of time. The first affected are the non-myelinated autonomic nerve fibres. This results in autosympathetectomy with consequent microvascular thermoregulatory dysfunction, medial artery calcification and arteriovenous shunting. Monckeberg calcification, or medial artery calcification, does not reduce arterial internal diameter. In the deeper tissues of the foot, hyper perfusion has been identified by non-invasive studies. Due to the microvascular dysfunction and arteriovenous shunting, a relative epidermal ischemia has been recognized by transcutaneous oxygen pressure measurements. Also anhidrosis due to autonomic neuropathy can cause cracking, dryness, and fissuring. Bacteria can easily enter through these defects in the epidermis. As opposed to popular belief, most diabetics have adequate circulation necessary for a cure. A hot and turgid foot is clinically noticed in patients in whom the autonomic dysfunction dominates. Other forms of neuropathy like sensory and motor become superimposed shortly after this. Thermal hyperalgesia and poorly tolerated tactile allodynia are the initial manifestations of sensory neuropathy. The objective loss of sensation and proprioceptive dysfunction are noticed as the thicker myelinated fibers are affected progressively. Axonal degeneration of the large motor myelinated fibers result in motor neuropathy. As a result of this, anterior crural muscles atrophy or intrinsic muscle wasting occurs. This causes foot deformities and altered foot biomechanics with foot pressure redistribution. The foot becomes insensitive and

deformed as the disease progresses. Claw toes, hammertoes, prominent metatarsal heads can be seen.

Screening for peripheral neuropathy

The evidence level at present for optimal screening method is limited. Nerve conduction studies correlate strongly with underlying structural changes in the nerves. They are the least subjective method. These studies are the most reliable single criterion standard for diagnosing peripheral neuropathy. However these studies are time consuming and costly. Hence they cannot be used as screening test. For this study we used a vibration sense testing at the medial malleolus level. This is based on a study by Perkins et al. Perkins et al found that 3 simple tests can be confidently used for screening of diabetic neuropathy. They include the 10-g SWME, superficial pain, and vibration testing by the on – off method. Vibration sensation is tested by placing a vibrating 128 Hz tuning fork over a bony prominence (medial malleolus in our study) and patients will have to raise their hand when they can no longer sense the vibration from the tuning fork. The examiner can usually feel the vibration from the tuning fork for 5 seconds longer. He usually feels it at the distal forefinger. Vibration is considered to be decreased if the examiner feels it for 10 seconds or more on his or her finger.

Vibration scoring	
Examiner feels the vibration	Duration
Present	<10 seconds,
reduced	>10 seconds
Absent	If patient can't feel any vibration

A study comparing the vibratory sensations at different sites was undertaken by Mitsuyoshi Takahara et al (J Diabetes Investig. 2014 Feb 12; 5(1): 90–93.). This was done using a retrospective database of 547 Japanese diabetic patients. The following sites were used to assess the vibratory sensation with a 128 Hz tuning fork: great toe, fifth toe, and medial malleolus. The study identified a significant association with one another (all $P < 0.01$). Although the vibratory sensations at the three sites had different associations with the pressure sensation and the ankle reflex, they showed similar C-statistics for the impaired pressure sensation and the disappeared ankle reflex. The study concluded that the vibratory sensations were strongly associated with one another at the three different sites. They could be clinically acceptable alternatives to one another in the assessment of diabetic peripheral neuropathy.

Peripheral arterial disease

Peripheral vascular disease, peripheral obliterative arteriopathy, and periperhal artery occlusive disease is defined as a narrowing of the arteries other than those that supply the heart or brain. Peripheral arterial disease commonly affects the tibial and peroneal arteries of the calf. It contributes to the development of foot ulcers in upto 50% of cases. Due to persistent hyperglycemic state, smooth cell abnormalities and endothelial cell dysfunction result in the peripheral arteries. Hypertension, smoking and hyperlipidemia are other common factors in diabetic patients and contribute to the development of PAD. The progression of peripheral artery disease, the presence of symptoms and the excess cardiovascular events associated with atherosclerosis are all the result of peripheral arterial disease. 27% of patients with peripheral arterial disease have a worsening of symptoms over a 5 year period with loss of limb occurring in 4%. For those with CLI, the outcomes are worse: amputations are necessary in 30% and death will occur in 20% within 6 months. There are no specific studies which have studied the natural history of PAD. But the data from prospective clinical trials of risk interventions show that the rates of cardiovascular events with diabetes and PAD are higher than those of their nondiabetic counterparts. One recent survey using the ankle brachial pressure index found a prevalence of PAD in people with diabetics >40 years of age to be 20% and those >60 years of age to be 29%. Abnormalities of vascular regulation and endothelial function are seen in most patients with diabetes. Various mediators play a role in endothelial cell dysfunction, but the most important

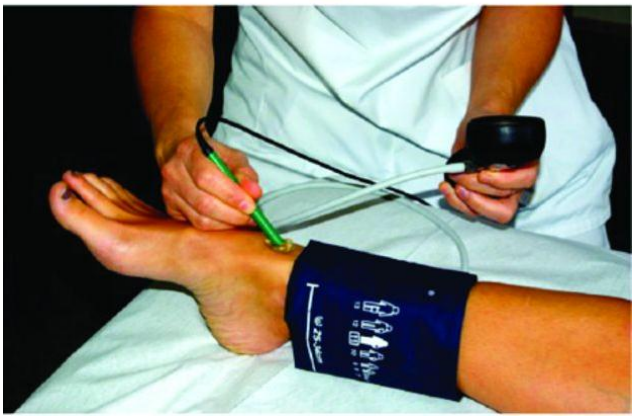
amongst them is the NO homeostasis. Various mechanisms contribute to loss of NO homeostasis, including hyperglycemia insulin resistance, and free fatty acid production. The hyperglycemia blocks the action of endothelial eNOS and boosts reactive oxygen species production. This impairs the vasodilator homeostasis fostered by endothelium. There is an amplification of the oxidative stress on endothelial cells as the glucose transport is not downregulated in diabetes mellitus. Insulin resistance also plays a role in the abnormal NO homeostasis in addition to hyperglycemia. There is an excess liberation of FFAs as a consequence of insulin resistance. These have adverse effects on the normal homeostasis, like inhibition of phosphatidylinositol-3 kinase, protein kinase C and production of reactive oxygen species. The sum effect of all these leads to the loss of NO homeostasis. Endothelial cell dysfunction along with the receptor activation for advanced glycation end products increase the inflammatory state of vascular wall. This is mediated in part by increase in the production of nuclear factor-kB, activation protein 1 and transcription factors. This leads to increased leukocyte chemotaxis, adhesion, transmigration and transformation into foam cells. This latter process is further augmented by increased local oxidative stress. Foam cell transformation is the earliest precursor of atheroma formation.

Screening for peripheral arterial disease

Currently the ankle brachial pressure index is accepted as the standard for screening for peripheral arterial disease. Amongst the various methods used to identify blood flow in the limb, the oscillometric method and the Doppler method are the commonly used ones.

A

B



We used the Doppler method to assess for ABPI in our study. This is done by tying a pneumatic cuff around the lower end of the leg and inflating it till the flow stops. This is followed by slow deflation till flow reappears. This is taken as the systolic BP.

The patient is kept in supine position at rest for about 10 minutes. After that, blood pressures are taken sequentially at the left arm, left leg, right leg and right arm. A handheld 5-10Mhz Doppler probe is used to identify the systolic BP.

Measuring the brachial pressure

The patient with the limb at heart level is kept in supine position. The cuff is secured around the arm. The brachial pulse is identified and the ultrasound gel is placed over it in the antecubital fossa. The probe of the handheld Doppler is placed over the ultrasound gel and the maximum intensity of signal is identified. The cuff is inflated slowly to a bit more than the expected systolic pressure. The signal from the handheld Doppler would disappear. The cuff is deflated carefully at a rate of 1mm/sec. The reappearance of the Doppler signal is identified. This is taken as the brachial systolic pressure and recorded.

Ankle pressure measurement

The ankle malleoli are identified and the cuff is placed proximal to it. The posterior tibial artery and dorsalis pedis is identified. Ultrasound gel is placed over the pulses. Usually the signal of the dorsalis pedis is found lateral to midline on the dorsum of the foot. The ultrasound probe is moved till the signal intensity is strongest. Again the cuff is inflated till the signal disappears. A similar technique as shown above is used.

The medial malleolus is identified and the posterior tibial artery is located posterior to it. The ultrasound gel is applied over it. The Doppler probe is used as described above.

Calculating the ABPI

An ABI is calculated for both the legs. The higher pressure of the two arteries at the ankle level is taken. That value is divided by the brachial artery

systolic pressure. The higher of the two brachial artery pressures are used to calculate the ABI.

Interpreting the ABPI

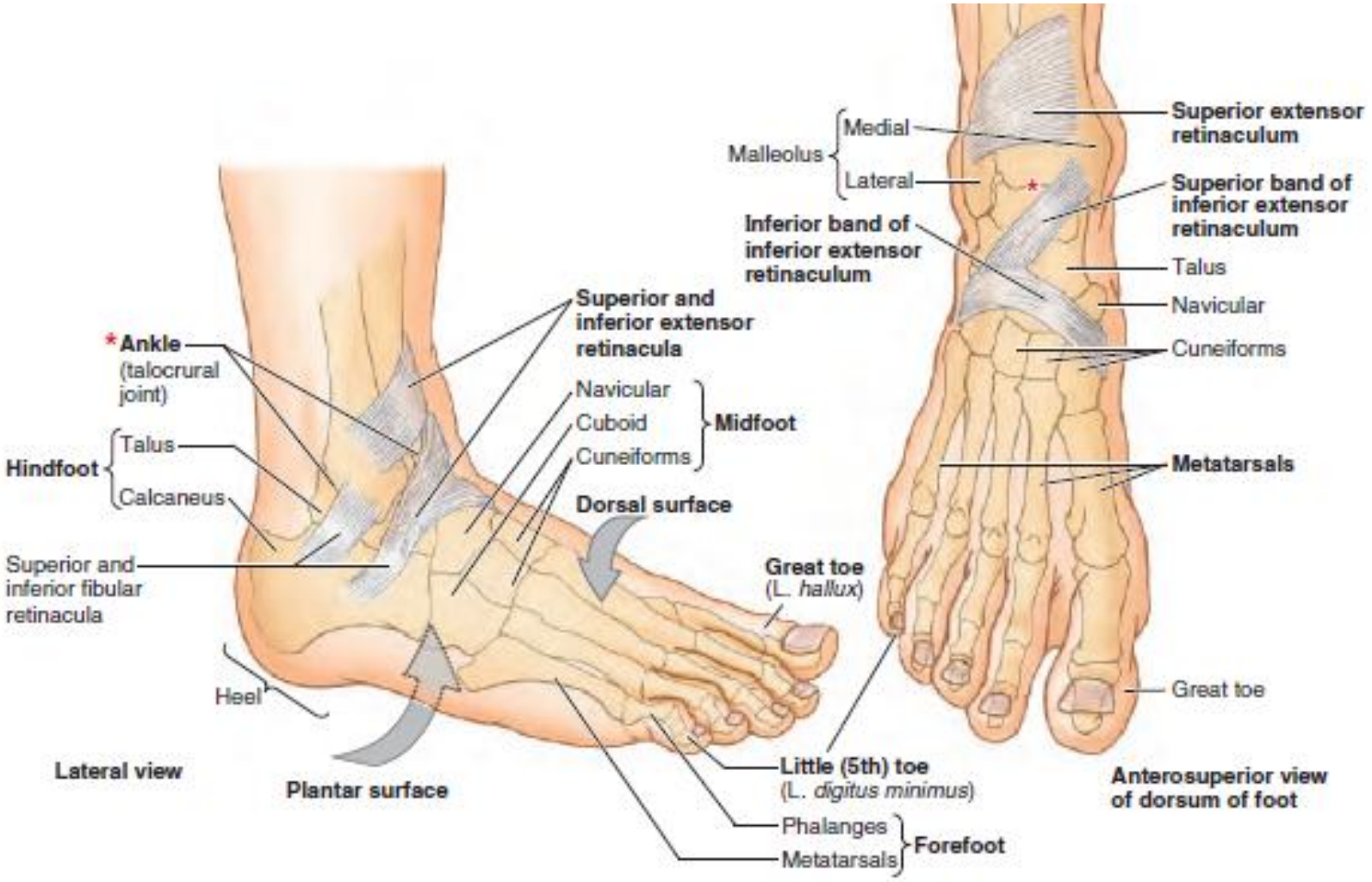
ABI Value	Interpretation	Recommendation
Greater than 1.4	Calcification / Vessel Hardening	Refer to vascular specialist
1.0 - 1.4	Normal	None
0.9 - 1.0	Acceptable	
0.8 - 0.9	Some Arterial Disease	Treat risk factors
0.5 - 0.8	Moderate Arterial Disease	Refer to vascular specialist
Less then 0.5	Severe Arterial Disease	Refer to vascular specialist

Anatomy of the foot

The foot is the part of the body distal to the ankle. It provides a platform to support the body. It has an important role in locomotion. The foot is composed of skin and soft tissues, bones, and neurovascular structures. The bones include fourteen phalanges, 5 metatarsals, 7 tarsals. There are three functional and anatomical zones: The hindfoot, midfoot, and forefoot.

- The hindfoot: talus and calcaneus.
- The midfoot: navicular, cuboid, and cuneiforms.
- The forefoot: metatarsals and phalanges.

The foot has various functions such as weight-bearing, weight distribution, ground contact (grip, abrasion), and containment. To accommodate for this the thickness, texture of skin, subcutaneous and deep fascia vary in the different regions of the foot.


Malleolus
Medial
Lateral
Superior extensor retinaculum
Superior band of inferior extensor retinaculum
Inferior band of inferior extensor retinaculum
Talus
Navicular
Cuneiforms
Metatarsals
Great toe
Little (5th) toe
(L. digitus minimus)
Phalanges
Metatarsals
Forefoot
Anterosuperior view of dorsum of foot
*Ankle
(talocrural joint)
Hindfoot
Talus
Calcaneus
Superior and inferior fibular retinacula
Superior and inferior extensor retinacula
Navicular
Cuboid
Cuneiforms
Midfoot
Dorsal surface
Great toe
(L. hallux)
Heel
Lateral view
Plantar surface

Skin and subcutaneous tissue

The skin of the sole of the foot is thicker and more sensitive than the skin on the dorsum of the foot. Oedema is more marked over the dorsum of the foot as the loose subcutaneous tissue deep to the dorsal skin allows fluid to accumulate. There is more fibrosis in the in the subcutaneous tissue in the sole as compared to other areas. Numerous fibrous septa divide the subcutaneous fat into small areas. This acts as a shock absorber. This is more pronounced over the heel. The grip function of the sole is brought about by the attachments of the skin ligaments to the underlying deep fascia. Hair is absent over the sole of the foot. There are numerous sweat glands present. The entire sole is sensitive.

Deep fascia of the foot

The thin deep fascia of the dorsal aspect of the foot continues proximally with the inferior extensor retinacula. The deep fascia is continuous with the plantar fascia (deep fascia of sole) over the posterior and lateral aspects of the foot. The plantar fascia has a weaker medial and lateral parts with a thick central part. A strong plantar aponeurosis is formed by the central thick part of the plantar fascia. It consists of dense fibrous tissue bundles which are arranged longitudinally. They invest the central plantar muscles. The palmar aponeurosis of the hand is similar to the plantar aponeurosis, but is denser, elongated and tougher. The parts of the foot are held together using the plantar fascia, thereby protecting the sole from injury and supporting the longitudinal arches of the foot. The plantar aponeurosis arises posteriorly from the calcaneus and functions as a superficial ligament. The collagen fiber bundles are arranged longitudinally and

divide distally in to five bands that becomes continuous with fibrous digital sheaths that enclose the flexor tendons that pass to the toes. The aponeurosis is reinforced by transverse fibers of the aponeurosis at the anterior end of the sole and forms the superficial transverse metatarsal ligament inferior to the heads of the metatarsals.

Figure: Subcutaneous tissue

Figure: Arrangement of the deep fascia

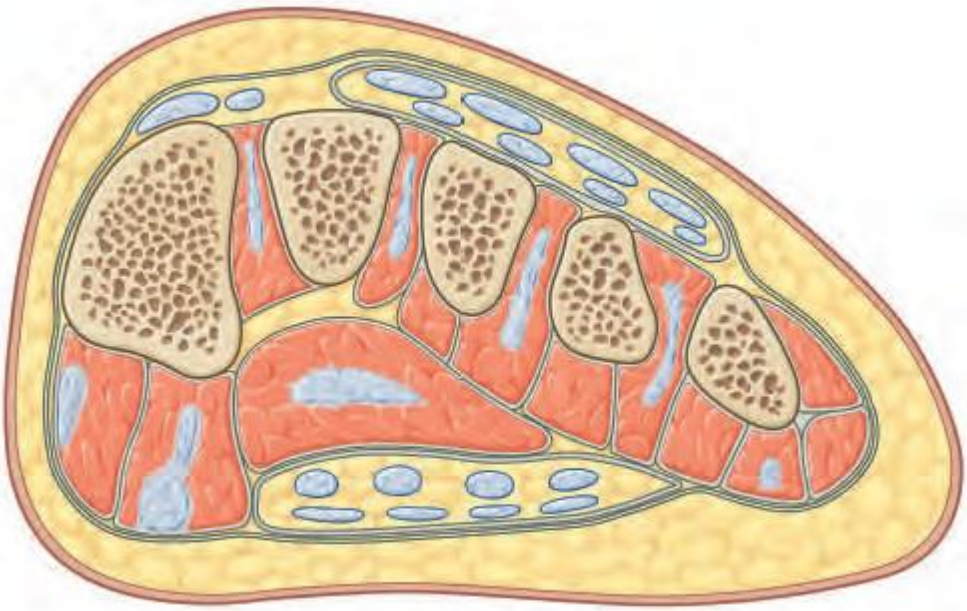
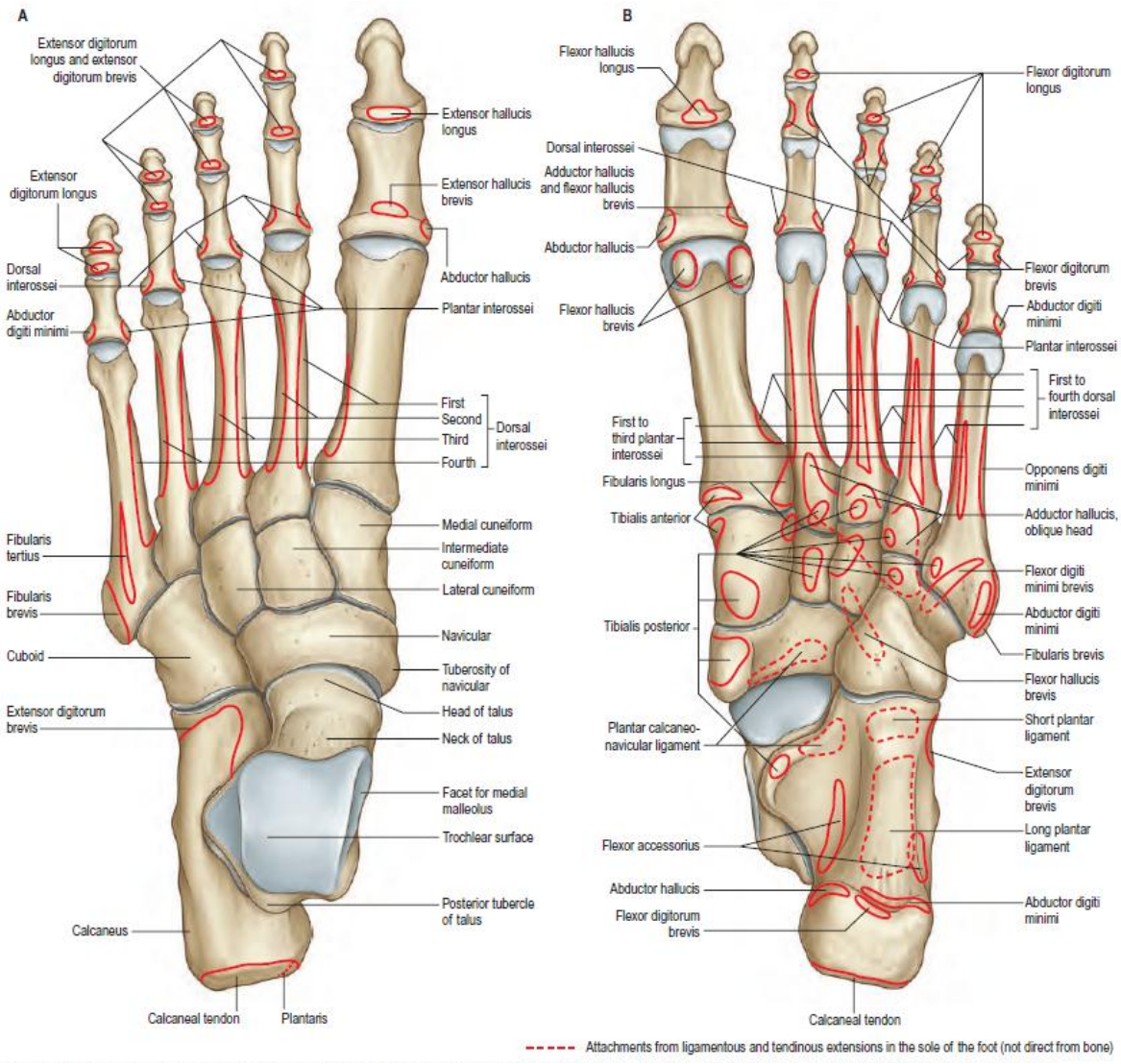


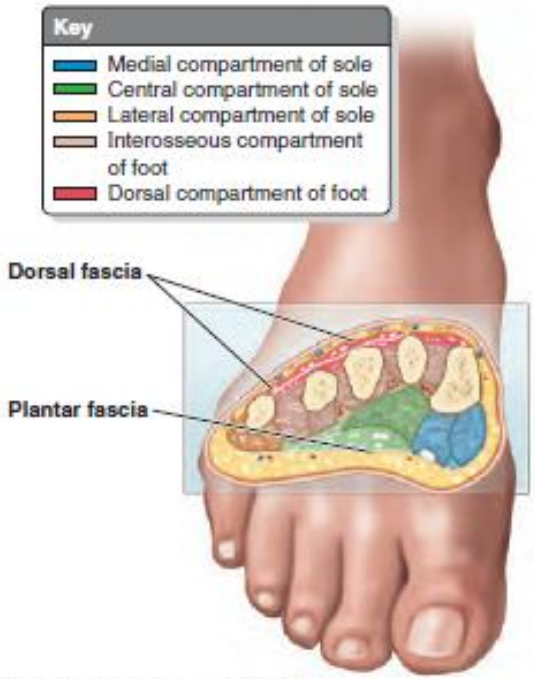
Figure: Skeleton of the foot



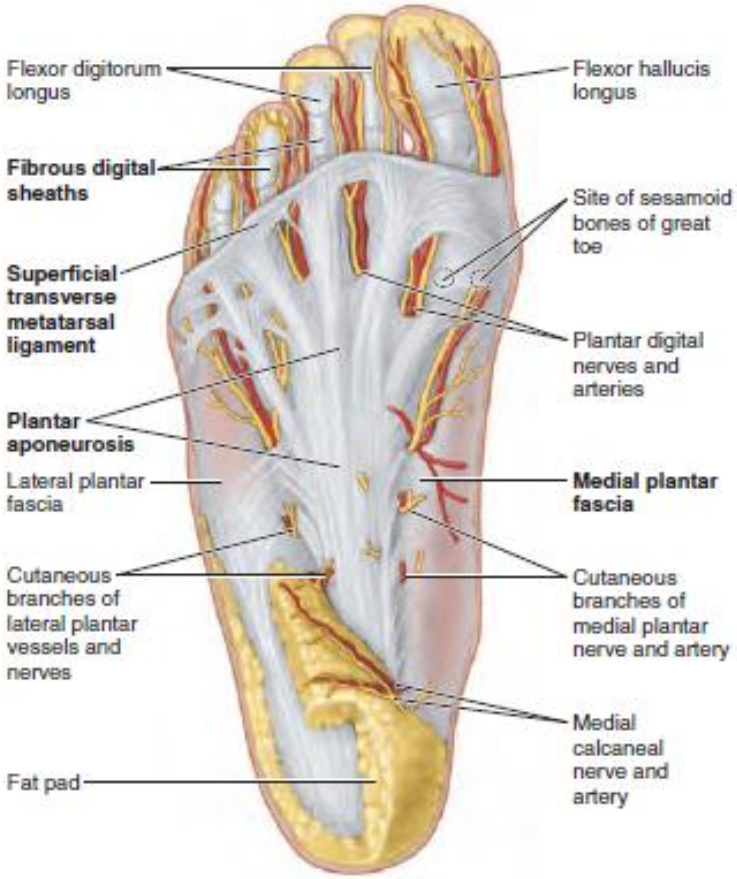
There are twenty muscles of the foot. Of them, 2 are on the dorsal aspect, 4 are in the intermediate position and remaining 14 are located on the plantar aspect. The foot is divided into the various compartments. The medial compartment contains the abductor halluces, flexor halluces brevis, tendon of the flexor halluces longus, medial plantar nerve and vessels. The central compartment of the sole is covered by the dense plantar fascia superficially. It contains the flexor digitorum brevis, the tendons of the flexor halluces longus, flexor digitorum longus, quadratus plantae, lumbricals, adductor halluces, lateral plantar nerve and vessels. The lateral compartment of the sole is covered superficially by the thinner lateral plantar fascia. The flexor digiti minimi brevis and abdcutur digini minimi are located in this compartment. The interosseous compartment of the foot is the fourth compartment. The dorsal and plantar interosseous fascia covers it. The interosseous muscles, deep plantar and metatarsal vessels and metatarsal bones are contained within it. The fifth compartment, the dorsal compartment lies between the dorsal fascia of the foot and tarsal bones, dorsal interosseous fascia of midfoot and forefoot. The extensor halluces brevis and extensor digitorum brevis muscles are located on the dorsum of the foot.

The plantar muscles act in coordination to support the stance, maintain the arches of the foot.

(A) Fascia of dorsum of foot (anterolateral view)



(C) Transverse section of foot compartments (anterior view)



(B) Sole of foot (plantar aspect) (inferior view)

Blood supply

The blood supply of the skin around the ankle includes anteriorly the anterior lateral and anterior medial malleolar arteries from the anterior tibial artery. Posteriorly the branches of the posterior tibial artery which is the posterior medial malleolar branch are involved. The posterior lateral malleolar branches from the fibular artery and thefasciocutaneous perforators from the posterior and anterior tibial and fibular arteries also supply. The heel is supplied medially from the medial calcaneal branches of the posterior tibial artery. The Lateral calcaneal branches from the fibular artery and lateral tarsal artery supply the skin of the lateral heel. The skin of the foot derives its blood supply from the branches of the dorsalis pedis, posterior tibial and fibular arteries. The skin over the dorsal aspect of the foot is supplied by the first dorsal metatarsal artery and the dorsalis pedis artery. There are smaller contributions from the anterior perforating branch of the fibular artery and marginal anastomotic arties on the lateral and medial borders of the foot. The perforating branches of the medial and lateral plantar arteries supply the skin over the plantar surface of the foot. The cutaneous branches of the common digital arteries supply the skin of the forefoot. The plantar and dorsal venous arches draining the cutaneous aspect of the foot into the medial and lateral marginal veins. These medial marginal vein continues as the long saphenous vein and the lateral marginal vein continues as the short saphenous vein. A superficial venous network is formed on the plantar aspect of the foot in the intradermal and subdermal layers which again communicate into the medial and lateral marginal veins. Veins from a deep venous network

accompany the medial and lateral plantar arteries. There is a unique bidirectional venous flow in the foot. But, the flow is from plantar to the superficial veins when valves are present. The superficial and deep veins of the lower limb carry the blood from the foot. Lymphatic vessels that accompany the long saphenous vein drain the superficial aspect of the foot except the superficial posterolateral area. This area is drained by the lymphatic vessels accompanying the small saphenous vein into the popliteal lymph nodes. There are deep lymphatic vessels accompanying the posterior tibial, dorsalis pedis and fibular arteries. These vessels drain into the popliteal lymph nodes.

Diabetic foot

A diabetic foot refers to a foot suffering any pathology that results as a direct cause of diabetes mellitus or chronic/long term complication of diabetes mellitus. Diabetic foot syndrome refers to the several characteristic pathologies of the diabetic foot such as neuropathic osteoarthropathy, diabetic foot ulcer and infection.

In our study we are dealing with diabetic foot infections which usually includes: Cellulitis, Deep-skin and soft tissue infections; Acute osteomyelitis; Chronic osteomyelitis.

Cellulitis

They are characteristically flat, non-raised skin lesions which are tender and erythematous. Lymphangitis usually accompanies it. Group A streptococcal

infection is the usual culprit. Staphylococcus aureus are usually associated with bullae, however even group A streptococci may cause it. Wound exudate or ulcer is usually not present.

Deep-skin and soft-tissue infections

Usually the patient is acutely ill, with excruciating induration of soft tissues of the extremity. The presence of wound discharge is usually not seen. The affected area may show crepitations in infections that involve the anaerobes. Compartment syndrome or clostridial infections is ususally indicated by extreme pain and tenderness.

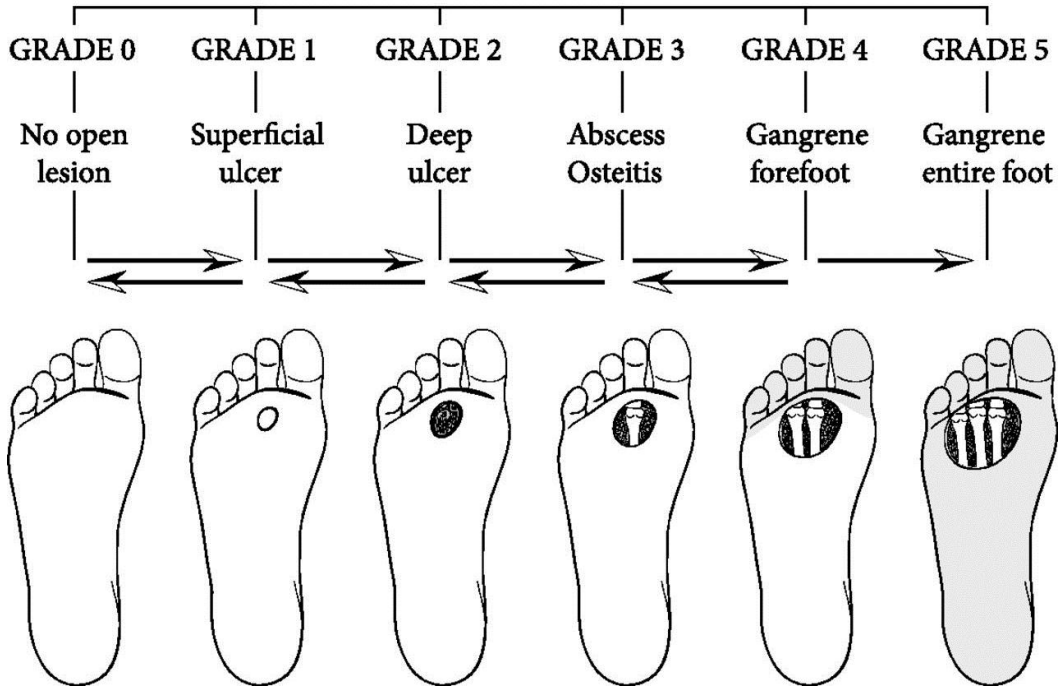
Acute osteomyelitis

The involved bone is usually painful, unless peripheral neuropathy is present. Regional lymphadenopathy and fever are usually absent in cases of small bone involvement.

Chronic osteomyelitis

There is usually foul smelling discharge from the deep penetrating ulcers and sinus tracts. These sinuses can be located between the toes or on the plantar surface of the foot. The heels, shins or medial malleoli are not usually involved. Lymphangitis is usually absent and the patient is only mildly febrile.

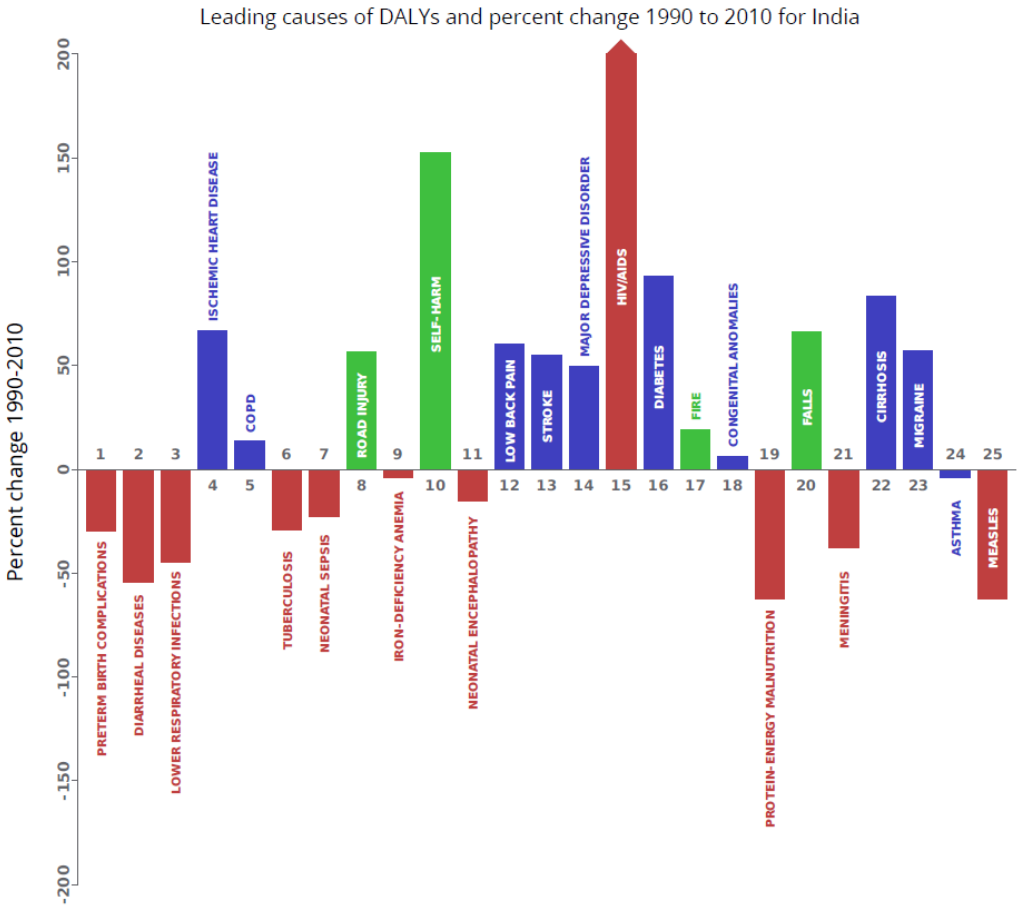
Wagner grading system for Diabetic foot infections



Grade	Characteristic
0	Intact skin
1	Superficial ulcer of skin or subcutaneous tissue
2	Ulcers extend into the tendon, bone, or capsule
3	Deep ulcer with osteomyelitis or abscess
4	Gangrene of toes or forefoot
5	Midfoot or hindfoot gangrene

Economic burden of diabetic foot ulcers

The diabetic foot ulcers are huge socioeconomic burden on the population of India. It accounts for an increasing number of DALYs(Disability adjusted life year).



The above graph from the Global Burden of Disease Study 2010 (GBD 2010) conducted by the University of Washington shows the Diabetes to be a leading cause of DALY in India. The following table from a study conducted by Pendsey (Pendsey S P. Indian Scenario; the Diabetic Foot in complications of Diabetes in Indian Scenarion; Nidus 99 Diabetology Initiative in Diabetology:

Proceedings; 1.) shows the direct cost of treatment of complete healing of diabetic foot. This does not include the indirect and incidental costs to the patient.

Type of lesion	**Treatment**	**Direct cost (INR)**
Neuropathic ulcer	Ambulatory	Rs 3696
Infected neuropathic foot	Ambulatory	Rs 10890
Advanced Diabetic foot	Salvage	Rs 71200
Advanced Diabetic foot	Limb amputation	Rs 63360
Advanced Diabetic foot	Salvage then Amputation	Rs 1,74,900
Neuroischemic foot	Bypass	Rs 1,29,360

The per capita income of the India is 93,231 rupees per year (Economic survey 2015-2016, Union Budget of India). So advanced diabetic foot can gobble upto a year's income for most patients. Hence further advancements are needed to bring about a drastic reduction in the healthcare costs.

Current management of diabetic foot ulcers

Debridement

One of the very important techniques in the preparation of wound bed includes debridement. It includes the removal of all nonviable tissues. This releases growth factors. These various growth factors act in concert to promote healing of the wound. The debridement also stimulates a previously nonadvancing wound edge.

All the necrotic, avascular and nonviable tissue are completely removed. The final end result is a red granular wound bed.

In cases where arterial insufficiency is suspected, aggressive debridement is usually postponed till a vascular examination is complete. Revascularization of the affected limb is completed before further debridement. Some of the commonly used methods of debridement include: Surgical, autolytic, chemical,

biologic and mechanical. There is selective debridement of the necrotic tissue by surgical, chemical and autolytic methods, whereas mechanical methods remove both the necrotic and viable tissue. Minimal tissue loss, avoiding loss of function of foot and deformities are the ideal aims of debridement.

Sharp debridement

This is the usually utilized method when the wound is significantly infected or necrotic. Also it allows the clinician to exactly determine the size and severity of the wound. It is the gold standard. The deeper tissues can be taken for culture when osteomyelitis is suspected. The scalpel blade in a 45 degree angle is used to remove all the necrotic tissues until the bed is healthy and bleeding. The wound edges are saucerized. Tissue nipper may also be used. If the foot has no sensation it can be performed in the office. But with intact sensation of the foot, the proper anaesthesia should be administered and debridement carried out in the sterile surgical theatre. A guillotine amputation will allow the control of infection promptly in cases where the disease threatens the patient's life. The wound can be subsequently closed after stabilization.

Enzymatic debridement

Enzymatic debridement uses the help of enzymes which are proteolytic in nature. Some examples include the collagenase, papain-urea, trypsin, papain, streptokinase-streptodornase. Once daily application is performed on the ulcer. This is covered using an occlusive dressing. Variable results have been obtained from clinical efficacy studies conducted using these agents. However most

studies comparing these agents with standard therapeutic treatment have not provided additional benefits. Slow softening of large eschars; wounds having tenuous arterial supply; decubitus ulcerations are some of the uses of these agents. To increase their effectiveness, a scalpel blade can be used to draw crosshatches. Due to their high cost and slow action, they are generally unpopular.

Mechanical debridement

Some examples of mechanical debridement include application of wet to dry saline gauze. The wet saline gauze is applied to the wound. The gauze dries over time. The necrotic tissue are incorporated into the gauze. The completely dried gauze is stripped. This removes the sloughed out tissue. However as this is nonselective, even the viable tissue may get damaged due to this technique. This will cause pain and discomfort to the patient. Large, highly exudative wounds are best treated with this method.

Some eccentric methods of debridement of wounds have been mentioned. Maggot therapy is one such method. They help debride the necrotic tissue, decrease bacterial load and stimulate wound healing. The effectiveness of this therapy in non-healing diabetic foot ulcers and pressure ulcers have been demonstrated in several clinical studies. However the high cost and low availability of medical grade maggots have not allowed the treatment to gain popularity. Low energy ultrasound mist is a new and upcoming technology. A low-energy, low-intensity ultrasound that is delivered through a saline mist to

the wound bed. This painless, non-contact, non-thermal energy facilitates: active cell stimulation; decreased bioburden; increased blood flow; cleansing and gentle debridement.

Figure: Maggot therapy

Figure: MIST therapy

Pressure Offloading

Offloading refers to the pressure relief on ulcers. This is usually used in plantar foot ulcers. Some of the methods to reduce the body pressure on the foot include half shoes, felted foam dressings, short leg walkers, total contact casing. The most effective method for offloading diabetic foot ulcers is the total contact casing.

A well moulded minimally padded plaster cast is applied to the affected limb. This allows the entire limb to take the weight distribution of the body instead of only high pressure points. As the weight is taken off the sole, the patient is able to ambulate easily, thereby reducing the amount of oedema. Presence of oedema impairs healing. These total contact casts should be changed atleast weekly. As the patient is unable to remove the cast easily by himself, this

method is effective. Some drawbacks include the amount of time, learning curve and skill to apply these casts. Also the cast may irritate the skin. The treating physician is unable to assess the wound daily. Some alternate methods include the scotchcast boot. The main advantage of this type of cast is that it is removable, and allows regular inspection and redressing of the wound. Half shoes help to reduce the pressure on a particular part of the foot.

Figure: Half shoes

Felted foam dressings are a light weight method to allow offloading. A piece of felt foam pad cut to the size of the foot is taken and an opening is made over the ulceration and applied. This allows for pressure relief to be customized from person to person. A tape or rubber cement is used to attach the pad to the patient's skin preventing migration and patient compliance. The aperture allows

for wound assessment. It must be changed every 2 weeks to allow for the integrity of the dressing.

Figure: Felted foam dressing

Patients with DFUs are asked to limit their everyday activity. This allows for faster healing of the ulcer. Also due to the bulky nature of these casts, patients are typically less active than their usual self.

Treatment of infection

The ulcer in a diabetic foot acts as a portal of entry for bacteria. This leads to wound infection. The clinical appearance and signs such as pain, oedema,

erythema, warmth and tenderness allow for the diagnosis of infection. High bacterial loads impede wound healing process. They slow down spontaneous healing and surgical closure. Infection can range in severity from superficial cellulitis to necrotizing fasciitis and systemic toxicity. Even mid cellulitis can rapidly progress to a limb threatening infection. Hence utmost care and prompt treatment should be given.

The usual organism involved are due to the aerobic gram positive cocci and aerobic gram-negative organism, Anaerobes are also involved. Swab cultures from clinically uninfected ulcers will only show colonizing flora. Initially empirical therapy is started and revised after the swab culture results are obtained.

Plain radiographs may show signs of osteomyelitis like periosteal reaction, cortical bone destruction and focal osteopenia. But these changes can be seen only after 10-14 days of osteomyelitis. MRI, CT may hasten the diagnosis of osteomyelitis by they are not routinely used due to high cost. They can perhaps be used in the care of chronically infected ulcers.

The management of clinically infected ulcers requires thorough debridement of all necrosis, drainage of purulent collections and appropriate antibiotic therapy. The bacteria usually involved in non-limb threatening infections are streptococci and staphyhlococcus. Limb threatening infections are frequently due to polymicrobial infections.

Mild cellulitis is treated on an outpatient basis. The patient is started on oral antibiotic therapy. The empirical therapy may start with a first generation cephalosporin. Moderate-severe infections have erythema that exceeds by 2 cm the wound margins. Foul smelling discharge, fever are the usual accompanying signs. These are treated with hospitalization and IV antimicrobial medications. Usually 2 weeks therapy of IV antibiotics is necessary. Osteomyelitis requires the removal of the offending bone. Antibiotics are usually not continued after clinical evidence of infection has come down.

The foot vascularity determines the efficacy of the antibiotics. In feet with tenuous blood supply, there is improper distribution of the antibiotics leading to inadequate infection control. Hence revascularization procedures are an integral component of management of diabetic foot.

Wound care

Meticulous and diligent use of dressings is necessary to ensure the excellent management of diabetic foot ulcers. The clean and moist environment for wound healing has been established as the ideal. Due to the moist environment there is less desiccation of tissues, cell death. The interaction of growth factors with target cells is facilitated because of the moist environment. Also angiogenesis is accelerated.

Wound bed preparation

Wound bed preparation refers to the conversion of the chronic wound into an acute healing wound. This is brought about by changing the molecular and cellular environment. This is a new concept in the management of the wound.

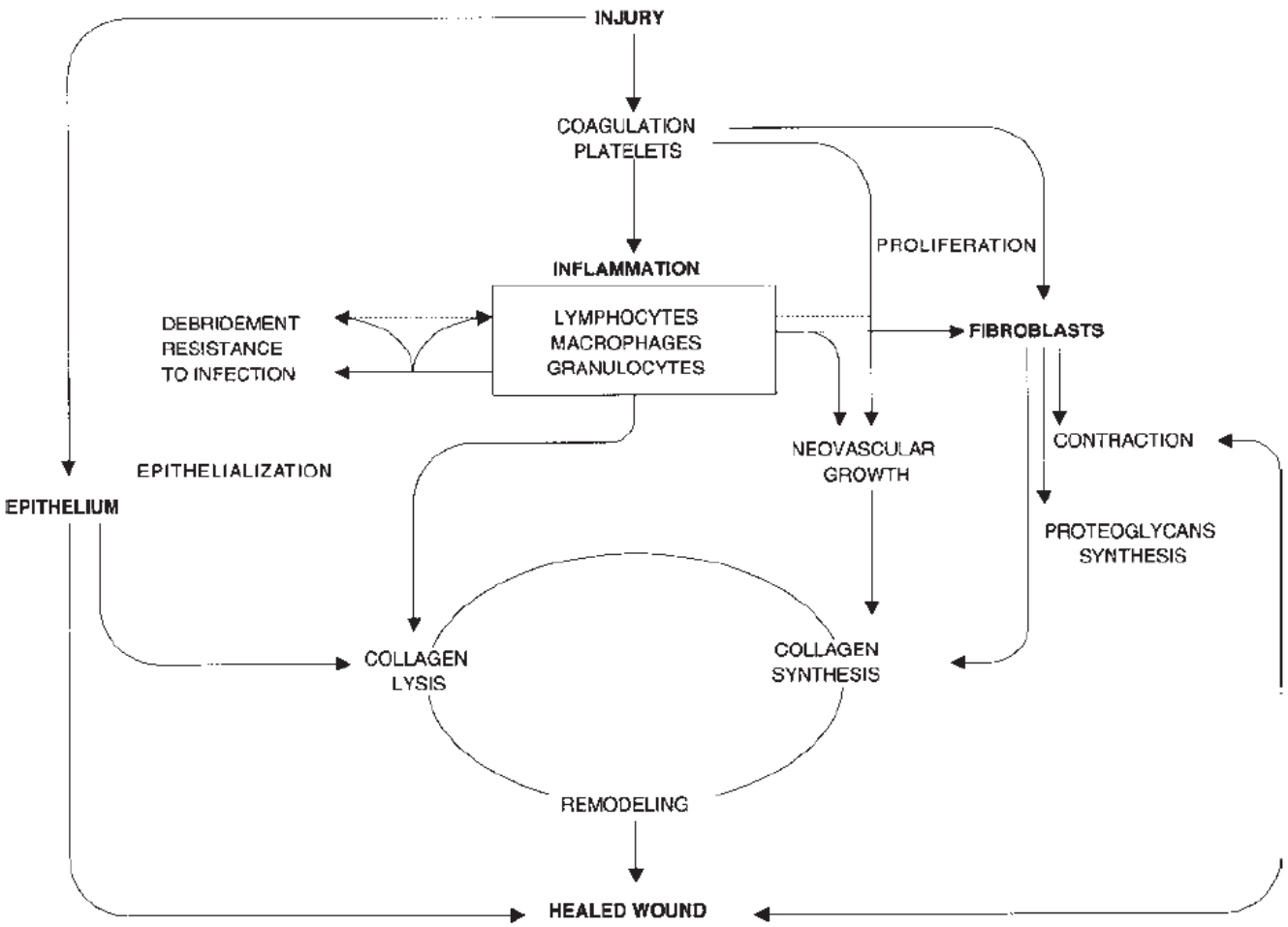
The methods include established concepts like surgical debridement which remains the gold standard, and wet to dry dressings. Newer dressings like hydrogels, collagen, photolytic enzymes, alginates etc have not been proven in clinical studies to improve the wound healing rates as compared to traditional methods.

Wound healing

Phases

There are four phases in the process of healing of acute wounds. They include: inflammation, epithelialization, proliferation and remodelling. These phases are a cascade and not a series of steps. This sequence of repair is rapid in primary healing where the superficial and partial thickness skin are only involved. Full thickness skin loss and subcutaneous tissue involvement are associated with slow wound healing.

Diagram of wound healing



Chronic wound healing

Chronic wound healing is associated with secondary intention model. Chronic wound can be defined as one that has "failed to proceed through an orderly and timely process to produce anatomic and functional integrity; or proceeded through the repair process without establishing a sustained anatomic and functional result".

Orderly refers to the sequence of the phases of wound repair described above. Timely refers to the expeditious progression of the above phases to heal the wound.

Examples of chronic wounds include: ischaemic arterial ulcers, diabetic vascular and neuropathic ulcers, venous ulcers, vasculitis ulcers, rheumatoid ulcers and pressure ulcers.

There are several factors which cause an interruption in the inflammatory phase of repair. These include ischemia, inadequate perfusion, oxygen reperfusion injury, free radicals, stimulus for repair and balance of inflammatory cytokines and proteases.

Stimulus for Repair

Acute and chronic wound healing primarily differ in the simulating factor for repair of the wound. The vascular disruption in an acute wound initiates the haemostasis process and thus the wound healing cascade. Whereas in chronic wounds such a stimulus is gradual in onset and may come from within.

Ischemia and Inadequate perfusion

In an acute wound, there is tissue ischemia as a result of the trauma. When the wound is overwhelmed by this ischemia it acts as a barrier to the wound healing by stopping the cascade of healing. Due to small thrombosis in the tissues, autonomic nervous system activation results in the constriction of vessels and free radical production causing oxygen reperfusion injury. This results in ischemic tissues with a long and arduous course of healing. Deficiency of venous and arterial system interferes with the homeostasis and coagulation cascade. In chronic wounds, the chemokines which stimulate the angiogenesis are suppressed and the process retreats from wound edge. As a result the wounds heal slowly and new blood vessels drop out. This causes an ischaemic wound site. Inflammatory mediators released as a result of this tissue ischemia triggers leukocyte infiltration, oxygen species formation and causing further tissue damage. Because of decreased pliability of vessels and capillary plugging, the ischemia further progresses. Due to this the WBCs are unable to enter the wound site. This prevents the inflammatory phase of wound healing from progressing. Arterial ulcers have both macrovascular and microvascular disease. This leads to tissue ischemia. Stress, pain, smoking all lead to sympathetic over activity and cause vasoconstriction and decrease the perfusion to tissues. Calcium deposits seen in the microvasculature of the diabetic patients add to the ischemia causing anoxia and necrosis leading to gangrene as often seen in diabetics.

Free radicals

During the Electron transfer chain in the mitochondria, free oxygen radicals are generated. These radical species are used normally in cell metabolic processes and cannot escape from mitochondria. Due to their highly reactive nature, once they escape the confines of the mitochondria, they severely damage many cellular structures. Due to reperfusion of an ischemic limb, these radicals overwhelm the normal processes and cause damage to the cells.

Oxygen Reperfusion injury

Due to the resumption of blood flow to an ischemic site, excess free radicals are generated. These cause the production of xanthine oxidase from xanthine dehydrogenase which is a normal enzyme present in cells. This in turn compounds the injury by causing the release of further free radical species. All these cause damage to the microcirculation by causing severe damage to the endothelium by lipid peroxidation. Because of the reestablishment of blood flow, leukocytes like neutrophils infiltrate the area and lead to further damage.

Epithelialization phase

This is the second phase of wound healing. The wound edges changes and reepithelialisation of wound occurs. The denuded wound surface is restored. This serves as a protective mechanism preventing organisms from invading the body. These processes commence immediately after trauma.

Role of keratinocytes

Keratinocytes function to serve the process of reepithelialisation. The bulge area of the hair follicle contain epidermal stem cells. They differentiate into keratinocytes. These keratinocytes travel to the basal layers of the epidermis, and produce the epidermis. These keratinocytes transform into epidermal stem cells and differentiate to form the various layers of the epidermis. Insoluble proteins are synthesized by these cells, which cross link and produce a cornified layer the stratum corneum. This layer is a protective layer and keeps the water in and microbes out. Neighbouring cells for example the fibroblasts are also activated which release cytokines and start the wound healing cascade. The advancing keratinocytes clean the debris from the wound. They migrate and a new basement membrane is formed. The epithelial cells move as a compete sheet or in a leapfrog manner. In full thickness wounds, the dermal appendages are lost. This is an important source of keratinocytes. So only the wound edges can give rise to keratinocytes in full thickness wounds. The migratory epithelial cells form an epithelial ridge. But due to low levels of oxygen in chronic wounds, the epithelial cells fail to migrate. Due to repeated attempts at repairing the wound, fibrosis and scarring occurs. After the epithelial cells resurface the wound, they differentiate and mature into scar tissue. The replaced scar tissue is friable and easily torn. Also the tensile strength is reduced compared to the original. So the replaced scar should be carefully managed as even mild trauma can cause infection and oedema.

Epithelialization phase in chronic wounds

If the process of reepithelialisation is arrested, it results in a chronic wound. Biopsies taken from chronic ulcers showed mitotically active and hyper-proliferative epidermis. The wound edge keratinocytes are only partially activated. These cells are partially unresponsive to cytokines. Due to a lack of a moist, oxygen-rich, nutritious tissue base, the keratinocyte migration is delayed. In large wounds, the reepithelialisation is predictably delayed due to the increased surface area. Some new technologies include development of human skin equivalents. These are combined with the own keratinocytes of a patient to reconstruct the skin.

Proliferative phase

It starts 3 to 5 days post-injury and continues for 3 weeks in wound healing by primary intention. In the proliferative phase: angiogenesis, collagen synthesis, and finally wound contraction take place. The repair cells require high oxygen and nutrition demands. The phagocytes at the wound work to control infection in the open wound. This raises the temperature of the tissues.

Integrins are receptor found on migrating cells that serve in cell adhesion and wound healing signalling. Growth factors like AGFs, TGF-B,TNF-A, IL-1 are produced by the proliferating cells to promote cell migration, proliferation, angiogenesis and synthesis of extracellular matrix.

Angiogenesis

Neovascularization begins in the inflammatory phase. It starts as new capillary buds arise and permeate the wound from nearby vessels. The newly synthesized capillaries form a network of new vessels and fill the tissue defect. Passage of fluids from intravascular space of the new vessels are easy as they have loose junctions and gaps in the endothelial lining. This allows all the nutrients necessary for wound healing to be made available. The granulation tissue, named after the granular appearance of the new capillary loops, first appears as pink pale buds. As it progresses, it attains a beefy red appearance. This new granulation tissue must be protected as damage to it will lead to excessive deposition of collagen.

Fibroblasts

Fibroblasts are an integral part of the biosynthesis of collagen. Collagen forms the main component of extracellular matrix. The ECM cements the wound healing process. The collagen is secreted to fill the wound and the fibroblast activity is reduced. Initially procollagen is synthesized by the coiling of 3 polypeptide chains. This procollagen is extruded from fibroblast into extracellular space, where it transforms into the tropocollagen molecule by cleavage of the procollagen. Collagen fibril is formed by the tropocollagen convolving with other similar molecules. These collagen fibrils along with the ground substance form the ECM which acts as a scaffold for tissue repair. ECM includes not only collagen but also elastin, fibronectin, reticulin. During the later

stages of wound healing the collagen crosslinkage occurs. Ground substance which is an integral part of the ECM is made up of water, salts and GAGs.

Myofibroblasts which differentiate from fibroblasts, help to contract the wound size thereby reducing the surface area of the wound.

Difference between a chronic wound and an acute wound

The proliferative phase of chronic wound healing is different from an acute wound. Some of the differences include:

Fibroblast senescence

As the fibroblasts double to heal the wound, they start to become senescent after a few population doublings. These cells do not actively replicate and also produce collagen necessary for healing.

Fibronectin composition

In chronic wounds, fibronectin, a matrix protein was partially degraded. In acute wounds it remained intact. These fragments increase the activity of matrix proteases. This leads to breakdown of connective tissue. Due to imbalance of synthesis and breakdown of connective tissue, healing becomes delayed.

Chronic wound fluid

Researches have elucidated the difference between fluid collected from chronic wounds and acute wounds. The fluid is mitogenic in acute wounds whereas it is inhibitory to regenerative cells. In chronic wounds, proteases and

inflammatory cytokines are present at increased levels as compared to the growth factor levels.

Protracted inflammatory and proliferative responses

The levels of proinflammatory cytokines and MMPs are increased in wounds that suffer repeated trauma and infection.

Dead space

Tissue gap is seen in full thickness ulcers which lengthens the time form proliferation as more tissue needs to be synthesized. The substrates required for new tissue growth are protein, vitamin C and zinc. When these substrates are inadequate, for example due to increased levels of bacteria, it leads to stalling of the wound growth process.

Remodelling phase

This phase starts as granulation tissue forms. It is the longest phase and lasts for one to two years. There is an increase in the tensile strength of the wound from about 10% in the early stage to 80% in the final scar.

There is a gradual and continuous change in the composition of ECM and collagen in the wound. The main change is the replacement of Type III collagen to Type I collagen. The Type I collagen is stronger than Type III collagen due to strong cross linkages and bundle construction. They are laid down in an organized fashion parallel to the lines of tension. The vascularity and cellularity of the granulation scar tissue reduces gradually.

Normotrophic scarring results in a scar that is flush with the surface. This occurs due to the progressive reduction of the vascularity and cellularity of the scar making it less bulky.

Remodelling in chronic wounds

The entire remodelling process is dysregulated. The optimum equilibrium between collagen synthesis and lysis seen in acute wounds are out of balance here. So there is no endpoint to the healing process. This leads to exuberant granulation tissue, further inhibiting the movement of epithelial cells as they have to act against gravity. There is an overproduction of matrix proteins and fibrosis leading to an ugly scar formation.

Wound assessment

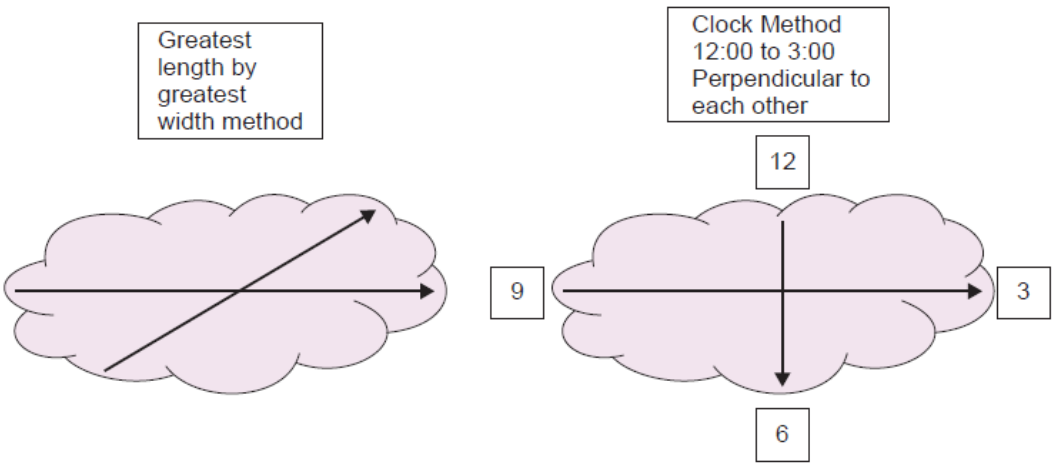
There are four wound assessment categories: Adjacent and periwound tissue appearance; Wound bed tissue appearance (color and texture); Wound edges; Exudate characteristics (odor, type, and quantity). Wound bed tissue is assessed by Surface area, undermining/tunnelling, depth and volume. They are direct indicators of healing.

Wound measurements

There are multiple methods of measuring a wound. They usually include the surface area, depth, volume and undermining. Some methods include linear measurements, wound tracings, and photography.

Linear measurements

The length and width of the wound are measured from edge to edge. They are multiplied to give the surface area. However these methods are not consistent as during repeat measurements the points chosen will not be constant.



The other method is the clock method where the measurements are taken perpendicular to each other.

Wound tracings

This is the method used in our study. A plastic household wrap or acetate sheet is placed over the wound and marked using a transparency marker. The sheet is removed and placed over a standard graph paper and the number of squares within the marked boundary are counted. This gives the surface area of the wound.

Wound photography

Serial photography of the wound under good lighting conditions and similar distance of the camera and wound will document the progression of the

wound. Disadvantages are it is more expensive. Photographic wound assessment tool can be used to keep an orderly record of the wound.

Measurement of wound healing

Multiple wound characteristics are used to monitor the wound healing process. The various standardized tools to measure wound healing include the Sussman wound healing tool (SWHT), Pressure Ulcer Scale for Healing (PUSH), Bates-Jensen wound assessment tool. In our study we use the Wound bed score developed by Falanga. Almost all wound healing scoring systems asses the following: location, shape, size, depth, edges, undermining, necrosis, exudate, surrounding skin characteristics, granulation tissue, and epithelisation. An adequately prepared wound would mean that it is free of infection, vascularized, free of fibrinous material, absence of scarring, and minimal exudate. These tools help us to assess that the wound bed is adequately prepared for high technology and advanced therapies such as bioengineered skin, negative pressure therapy. The following table is a comparison of the various wound assessment tools.

Table: Comparison of various wound scores

Wound Characteristics and Format	SWHT	PUSH	BWAT, previously PSST	PWAT	WBS	SCI-PUMT
Size	X	X	X	X		X
Depth or stage	X		X	X	X	X
Necrotic tissue	X	X	X	X	X	X
Granulation tissue	X	X	X	X	X	
Epithelial tissue	X	X	X	X	X	
Surrounding tissue Characteristics	X		X	X	X	
Exudate		X	X	X	X	X
Undermining and tunneling	X		X	X		X
Scoring Methods						
Likert scale			X	X	X	X
Subscales with total score	X	X	X		X	X

The tool used in this study is the wound bed score. This was developed by Falanga. In specifically addresses the wound bed preparation. It scores for wound bed appearance, wound exudate. It was revised in 2006 to add items relating to periwound tissue including healing edges, oedema, dermatitis, black eschar. Each component receives a score from 0 (worst) to 2 (best). The sum total of all score is calculated to give the final wound bed score. Better prognosis is linked to higher score. The total scores are divided into 4 quartiles. Scores 9 and below; scores between 10 and 11; scores of 12 and 14; scores from 14-16. The chance of healing increases by 22.8% for each quartile jump.

Wound bed score			
Characteristics	0	1	2
Healing edges	None	25%-75%	>75%
Black eschar	>25% of wound surface area	0%-25%	None
Greatest wound depth	Severely depressed	Moderate	Flushed
Exudate amount	Severe	Moderate	None/mild
Edema	Severe	Moderate	None/mild
Periwound dermatitis	Severe	Moderate	None or minimal
Periwound callus fibrosis	Severe	Moderate	None or minimal
Pink wound bed	None	50%-75%	>75%
Total WBS adds each individual score for each characteristic to give a total score			
The maximum possible score is 16			
The minimum possible score is 0			

Antimicrobial resistance

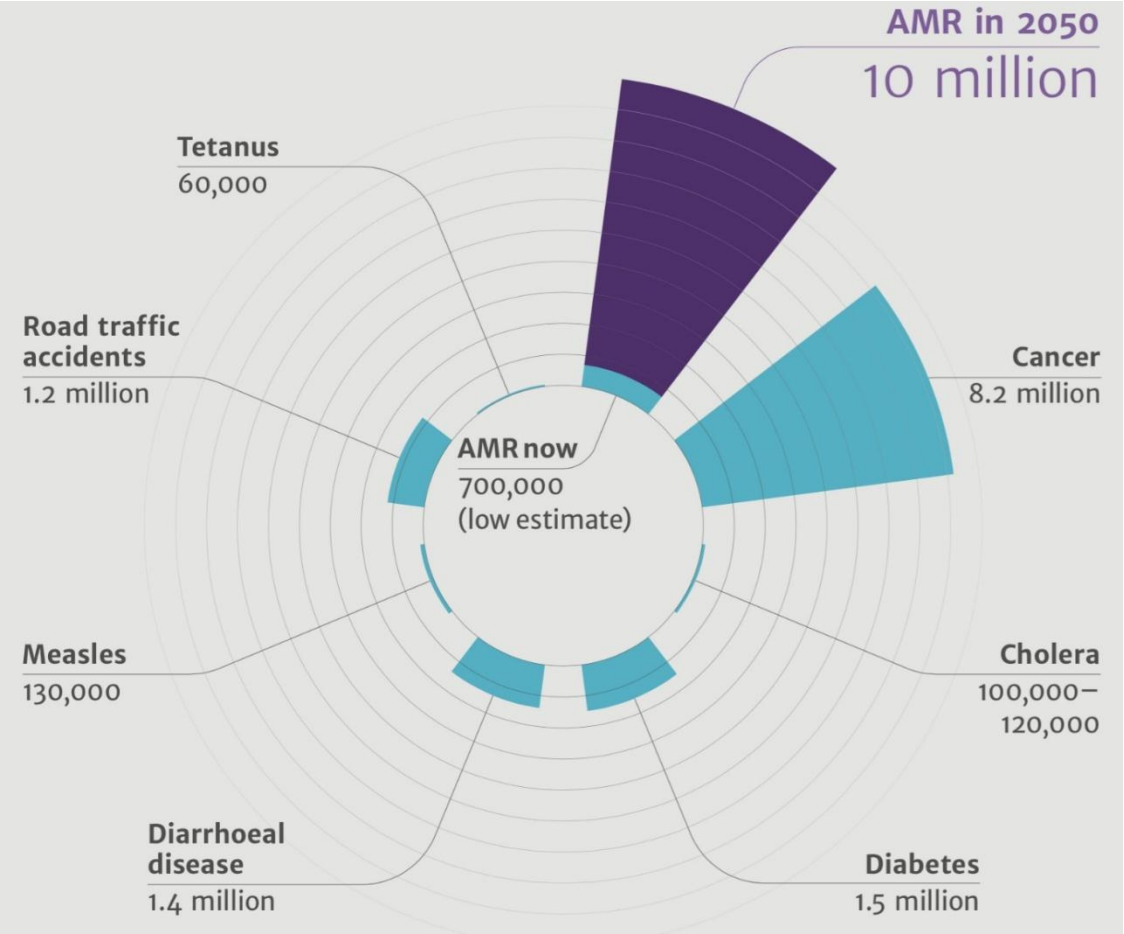
Antibiotic resistance refers to the resistance a microorganism to an antimicrobial medication that had been effective in treating or preventing infection caused by that microbe. There are various ways by which resistance occurs of which the following three are most important: natural resistance, genetic mutation, transference from one bacteria to other. These usually occur as a result of misuse of antibiotics. These microbes which become antibiotic resistant are difficult to treat and need alternative medications, higher doses which are costly and toxic. Antimicrobial resistance in rising and is estimated to cause millions of deaths every year. All classes of microbes including fungi, viruses, protozoa, bacteria develop resistance. The largest threat to public health is from the antibacterial resistance.

Antimicrobial resistance prevents the effective treatment of an ever rising range of diseases caused by various microbes. All sectors of the government and society must come together to combat this serious threat to global public health.

Resistance to antimicrobial agents in on the rise in all parts of the world and new mechanisms emerge and spread globally.

Since 2012, WHO has noted a slow decrease in the sensitivity to HIV drugs, this may lead to the requirement of more expensive drugs in the future.

Figure: WHO statistics for cause of deaths



IN 2013 alone, about half a million new cases of MDR TB was reported. Also now Extensively drug resistant tuberculosis has been reported in a hundred countries. This leads to longer and costlier treatment courses.

Other examples include the resistance to aretmisinin based combination therapies by falciparium malaria in the Greater Mekong subregion of China.

Most of the hospital acquired infections are caused by highly resistant bacteria such as MRSA (methicillin resistant Staph aureus). Gonorrhoea may

become completely incurable as resistance to the last known drug (third gen cephalosporin) have been detected in 10 countries.

Many standard medical treatments will fail or turn into high risk procedures if there are no effective antimicrobial agents. High health care expenditures, prolonged illness and greater chance of death are some of the consequences of antimicrobial resistance.

Some of the commonly used first line treatments are becoming rapidly ineffective. For examples, resistance against flouroquinolones in E.coli is very common. Also Staph aureus has become resistant to the first line drugs.

Now even the last resort antibiotics (carbapenem) for life threatening infections has spread to the entire world. In many countries there appear to be no tools even basic ones like systems to track and monitor the problem for the fight against antimicrobial resistance.

Biofilms

In chronic wounds, the bacteria form complex bacterial communities called biofilms. These bacteria constantly produce and secrete biofilms matrix which promotes colonization by more bacteria. The bacteria have the ability of "quorum sensing", which allows them to talk to each other by means of small organic compounds. This allows the bacterial colony to function as a multicellular organism.

In studies conducted earlier, it was discovered that polymicrobial biofilms impair the healing process as compared to a monomicrobial biofilm. These studies were initially conducted on rabbits, now the same has been proved on pigs, mice and humans. Researchers have also shown that physically disrupting the biofilm in a wound by mechanical debridement has allowed better penetration of topical antibiotics.

Bacterial colonies can change the biofilm environment. This lead to studies which dealt with bacteria based treatments to regulate the skin microbes. As an example, colonization of the nose with an ordinary commensal organism like S.epidermidis reduced the incidence of MRSA colonization in mice. These studies give a boost to nonantibiotic approaches to deal with microbes. Even prosthetic meshes which have endopeptidases impregnated to destroy biofilms have been proven to reduce infectious complications and increase the repair strength. Our present method of antibiotic prescription cannot continue for long due to rapid rise in antibiotic resistant bacteria. Hence novel methods need to be identified to treat infectious diseases.

Probiotics

Probiotics literally means “for life”. It refers to the normally present bacteria or yeast which has beneficial health effects when supplied in appropriate quantities. Some other terms similar to probiotics are prebiotics and synbiotics. Prebiotics refer to the indigestible molecules that are fermented by endogenous

bacteria. This helps to alter the gut microflora. Synbiotics refer to a combination of probiotics and prebiotics.

Probiotics have been known from ancient history but their importance, action and characteristics have become popular in recent times. Fermentation of milk products were done in Middle East and Asia since ancient times as their climates favoured it. These were used for gastrointestinal illnesses. Even before the mechanism of the probiotics were known, they were continued to be used for its various beneficial effects.

In 1899, bifidobacter was discovered by Henry Tissler in Paris, France. He studied that infants with bifidobacteria colonization in the GI tracts had fewer intestinal problems. Mitchnikoff was a scientist from Russia also studying in the Pasteur Institute in Paris. Certain normal bacteria like clostridia found in digestive tract which produced a type of intestinal auto-intoxication. These compounds include ammonia, phenols and other compounds. He found that rural Europeans lived longer than wealthier Europeans. This he inferred due to the usual consumption of fermented milk products. The lactic acid bacteria present in fermented milk was associated with anti-aging health benefits. "The prolongation of Life: Optimistic Studies" was published by Metchnikoff.

Metchnikoff received the Nobel Prize in 1908 in Medicine. He demonstrated that beneficial microbes can be used to replace harmful microbes to treat intestinal illnesses.

Alfred Nissle, isolated a new strain of E.coli during an outbreak of shigellosis. This new strain was isolated from faeces of World War I soldier who afflicted with shigella but did not suffer any illness. This strain was named Eshcericia coli Nissle 1917. This strain was used to treat diseases like shigellosis and salmonellosis and is still continued to be used today. At present, scientific evidence supports probiotic use for GI disorders like antibiotic associated diarrhoea, IBDs, PUD. Even conditions like atopic dermatitits, vaginal infections, ventilator associated pneumonia and hypercholesterolemia have been identified to have a role for probiotics.

The usual strains studied for probiotic activity include Saccharomyces, Enterococcus, Lactobacillus, bacillus, Bifidobacterium and streptococcus. In ancient times, probiotic remedies have been associated with good health but only now science has identified the mechanisms of action of these probiotics. Probiotics have been demonstrated in vitro to reduce the pathogenic strains in periodontal tissues.

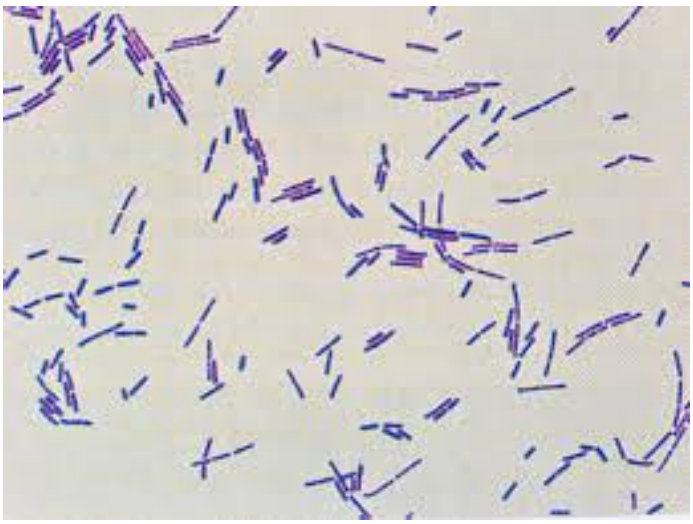
Probiotics improve the function and stability of epithelial barrier in the intestine. They increase the production of mucin, antimicrobial peptides, heat shock proteins, all which contribute to its beneficial effect. Human beta-defensin -2is an antimicrobial protein which is induced by the activity of probiotics. This and other antimicrobial proteins reduce colonization by pathogenic microbes. Probiotics release small molecules which bind with receptors like TLRs, NLRs and cause immunomodulation. This is beneficial to control infection.

There is a huge deficit in validated studies for evaluating probiotics. Also guidelines on probiotics which are evidence based need to be developed and accepted internationally. Thorough human studies must be conducted with clear endpoints to establish a clear role for them.

Lactobacillus

It is a gram positive organism. It is rod shaped and non-spore forming. The characteristic feature is to produce lactic acid. This is formed from metabolism of glucose. This organism is distributed widely in milk, milk products, manure, animal feeds. They are also used in the industry to produces yogurt, fermented vegetables and beverages, breads.

Figure: Lactobacillus



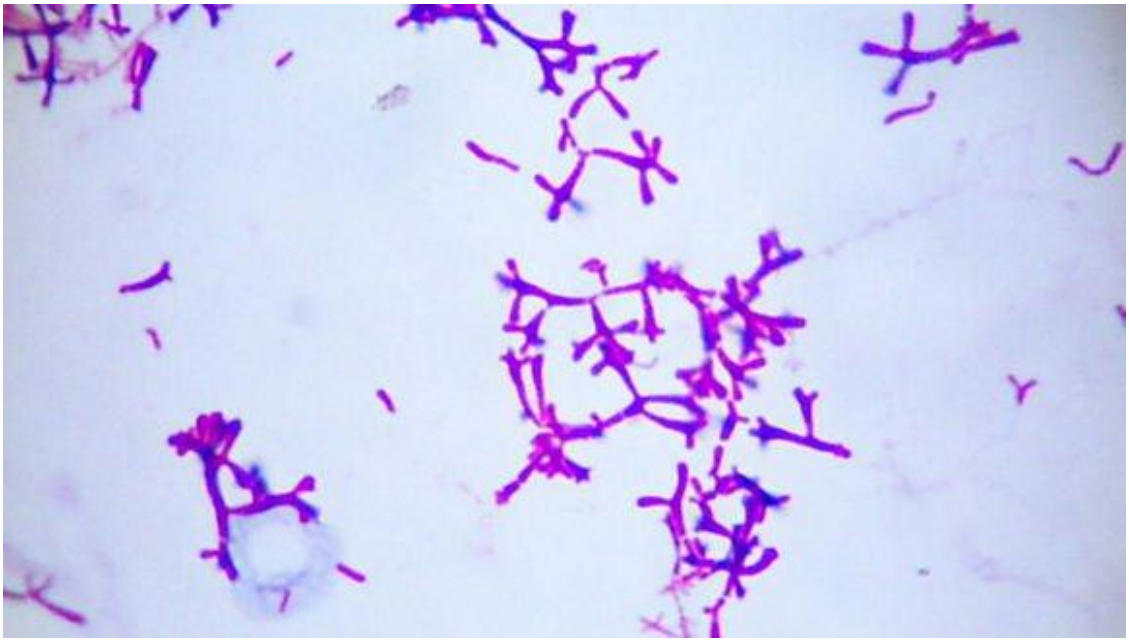
These bacteria are nonmotile. They can thrive in anaerobic and aerobic environments. The usually used bacteria in probiotics is the Lactobacillus acidophilus. This is a homofermentative bacteria where lactic acid forms almost

85% of the glucose metabolism products. They are common inhabitants of GI tracts of both animals and humans, vagina, mouth.

Bifidobacterium

It is a gram positive bacteria. It is not mobile, and branched. It is an anaerobic bacteria. They inhabit the mouth, GIT and vagina of mammals. It is one of the major type of bacteria in the colonic flora. They were earlier included in the lactobacillus genera, but later given their own after the 1960s.

Figure:Bifidobacterium



Some strains are important probiotics. The food industry is one where they are extensively used. They regulate the homeostasis of intestinal microflora. They have additional effects of inhibition of pathogenic bacteria that infect the GIT mucosa. They modulate immune responses, repress carcinogenesis, and

produce vitamins. They strengthen the gut mucosal barrier and reduce the levels of LPS.

These bacteria ferment the mother's milk in infants, thereby reducing the pH. This makes it an unfavourable environment for the bacteria to grow.

They are classified into four based on growth under different O2 conditions. They are microaerophilic, O2-tolerant, O2-sensitive, O2-hypersensitive.

Probiotic therapy for chronic wounds

Studies showing the importance of skin microbes and biofilms have prompted researchers to investigate the role of probiotics in chronic wounds. Few animal based experiments have been conducted. One study dealt with kefir extracts (which contains probiotics) on infected burn woundsin rats. This study showed better collagen formation and epithelialization than controls treated with silver sulfadiazine. Even oral probiotics were found to modulate interleukin levels and skin immune cell density in mice. Studies have shown that Lactobacillus acidophilus is inhibitory on majority of the burn wounds.

A study conducted by Peral et al in 2009, compared the healing of burns wounds using probiotics and silver creams. They showed almost equal benefits. The possible mechanism of action include disruption of quorum sensing and competitive inhibition of the offending bacteria. Fungal growth has also shown to be potentially reduced by probiotics. Some examples where the probiotics can

be used include grafts and implants. A topical patch has been developed which uses nitric oxide producing lactic acid bacteria to improve wound healing. This patch was studied in rabbits and positive results were obtained. Further research in probiotics, their mechanism of action, and potential delivery mechanism is the need of the hour.

Materials and methods

Study centre

Institute of General Surgery, Madras Medical College andRajiv Gandhi Government General Hospital

Study type

Prospective study

Study Duration: December 2015 to June 2016

Sample size: 40 patients

Hypothesis

Probiotic bacteria have a beneficial effect on the diabetic wounds

Aim

To study the effect of local application of probiotics on the healing of Diabetic foot ulcers

Objective

1. To compare the change in wound bed score in the test and control population
2. To compare the wound swab culture results in the test and control population

Patient inclusion criteria

Patients admitted with clinically infected diabetic foot to RGGGH Chennai with the following characteristics:

Age > 18 years

Age < 70 years

Diabetics with average RBS < 250

Ulcers involving the foot

Wound size more than 10 cm^2 and less than 60 cm^2

Exclusion criteria

Unstable vitals

Peripheral Arterial Disease

Peripheral neuropathy

Diabetic Ketoacidosis

Osteomyelitis

Probiotic suspension

The probiotic solution is prepared by dissolving 5 billion CFU of probiotic bacteria (lactobacillus plantarum manufactured by Pharmagenica Healthcare Inc) in 10ml of sterile water. This solution was applied to the wound at a volume of 1ml/cm^2 and dressed using cotton gauze and pad.

Assessment of wound characteristics

The patients are assessed daily for wound progression. Wound bed scoring system developed by Falanga is utilised to monitor the wound in an objective manner. Both the groups will be compared with respect to the wound bed score at day 1, day 7 and day 14 and the wound swab cultures and outcomes identified. Wound swab was checked for pathogenic bacteria. As per the policy of our microbiology department, swabs with normal commensal bacteria such a gram positive rods were reported as no growth. The results will be checked for statistical significance.

Wound bed score			
Characteristics	0	1	2
Healing edges	None	25%-75%	>75%
Black eschar	>25% of wound surface area	0%-25%	None
Greatest wound depth	Severly depressed	Moderate	Flushed
Exudate amount	Sever	Moderate	None/mild
Edema	Severe	Moderate	None/mild
Periwound dermatitis	Severe	Moderate	None or minimal
Periwound callus fibrosis	Severe	Moderate	None or minimal
Pink wound bed	None	50%-75%	>75%
Total WBS adds each individual score for each characteristic to give a total score			
The maximum possible score is 16			
The minimum possible score is 0			

Wound swab C&S

Wound swab cultures are taken at Day 0, Day 5 and Day 10 using a sterile swab and sent for microbiological analysis. They will be grown on nonselective media. As per the existing policy of our microbiology department, only

pathogenic bacteria would be reported whereas commensal bacteria like bacillus, would be reported as commensals.

Methods

Diabetic patients presenting with acute infected ulcers of the foot (below ankle) are taken up for surgical debridement on the day of presentation.

The size of their wounds are assessed by wound tracing and planimetrymethod. A household plastic wrap is placed over the wound. A transparency marking pen is used to mark the wound. The wrap is placed over a graph paper and the number of squares counted. Those with an area less than 60 $cm^2$are included in the study. The patients are screened for peripheral vascular disease by using ankle brachial pressure index. Only patients with ABPI >0.9 measured using Doppler methodwere taken up for the study. The patients are also screened for peripheral neuropathy at the medial malleolus using 128Hz tuning fork vibratory sense testing to exclude those with severe neuropathy.

Severely ill patients and those with diabetic ketoacidosis are excluded from this study.

The patients who consented to participate in the study were allocated into two groups based on the use of sequentially numbered, opaque sealed envelopes (SNOSE). The control group where the current regimen of sharp and chemical debridement at ward, cleaning and dressing, glycemic management and antibiotic therapy is given.

In the intervention group, in addition to the above, probiotic solution is applied daily during dressing. The probiotic solution if prepared by dissolving 5 billion CFU of probiotic bacteria in 10ml of sterile water. This solution was applied to the wound at a volume of 1ml/cm^2 and dressed using cotton gauze and pad. (This was based on the concentration used by Peral MC et al in their study on Burns patients using Lactobacillus plantarum).The patients are assessed for gylcemic control by taking RBS every 3 days and taking the average of the value. Patients whose average RBS is above 250 were removed from the study. The patients are assessed daily for wound progression. Wound bed scoring system developed by Falanga was utilised to monitor the wound in an objective manner. Wound swab cultures are taken at Day 0, Day 5 and Day 10. Both the groups will be compared with respect to the wound bed score at day 1, day 7 and day 14 and the wound swab cultures and outcomes identified. The results were be checked for statistical significance.

Analysis

The collected data was analysed for improvement in wound status between the either study groups and the change in swab status. The software used to analyse the data was SPSS software.

Results

Total of 40 patients were enrolled in the study but 4 were lost to follow up due to various reasons.

Control													
S. No.	**Name**	**Age**	**Sex**	**WBS Day 1**	**WBS Day 7**	**WBS Day 14**	**Wound swab C&S Day 1**	**Wound swab C&S Day 5**	**Wound swab C&S Day 10**	**RBS day 3**	**RBS day 6**	**RBS day 9**	**RBS day 12**
1	Ravi	25	M	8	10	13	Staph aureus	Staph aureus	No Growth	224	198	145	156
2	Ramnath	55	M	7	10	11	Pseudomonas	No Growth	No Growth	230	165	187	153
3	Eswari	60	F	9	11	13	Klebsiella	Klebsiella	No Growth	201	123	136	153
4	Loorthumary	48	F	8	9	12	Staph aureus	No Growth	No Growth	180	156	111	164
5	Arokiyamary	56	F	9	11	13	Staph aureus	Staph aureus	Staph aureus	175	178	123	178
6	Mariamma	55	F	8	10	12	Pseudomonas	No Growth	No Growth	146	196	187	193
7	Muniyamma	57	F	7	9	10	Klebsiella	Klebsiella	No growth	189	158	154	165
8	Vignesh	49	M	8	10	13	Staph aureus	Staph aureus	Staph aureus	153	200	132	128
9	Ramesh	62	M	9	11	15	Staph aureus	No Growth	No Growth	169	153	169	197
10	Badhushah	65	M	7	10	12	Staph aureus	No Growth	No Growth	201	168	180	153
11	Afzal	53	M	8	11	13	Pseudomonas	Pseudomonas	Pseudomonas	260	175	150	120
12	Kannan	52	M	8	10	14	Pseudomonas	Pseudomonas	Pseudomonas	302	146	130	102
13	Gokulakannan	45	M	9	12	12	Staph aureus	Staph aureus	Staph aureus	205	189	146	154
14	Ambiga	46	F	7	10	15	Klebsiella	Klebsiella	Klebsiella	260	150	159	163
15	Srinivasan	48	M	9	9	11	Pseudomonas	No growth	No growth	214	136	168	187
16	Ramachandran	69	M	8	10	14	Staph aureus	Staph aureus	No Growth	198	175	145	193
17	Arumugam	50	M	9	11	13	Pseudomonas	Pseudomonas	Pseudomonas	186	142	129	154
18	Gajendran	66	M	8	12	13	Staph aureus	Staph aureus	Staph aureus	173	126	136	130

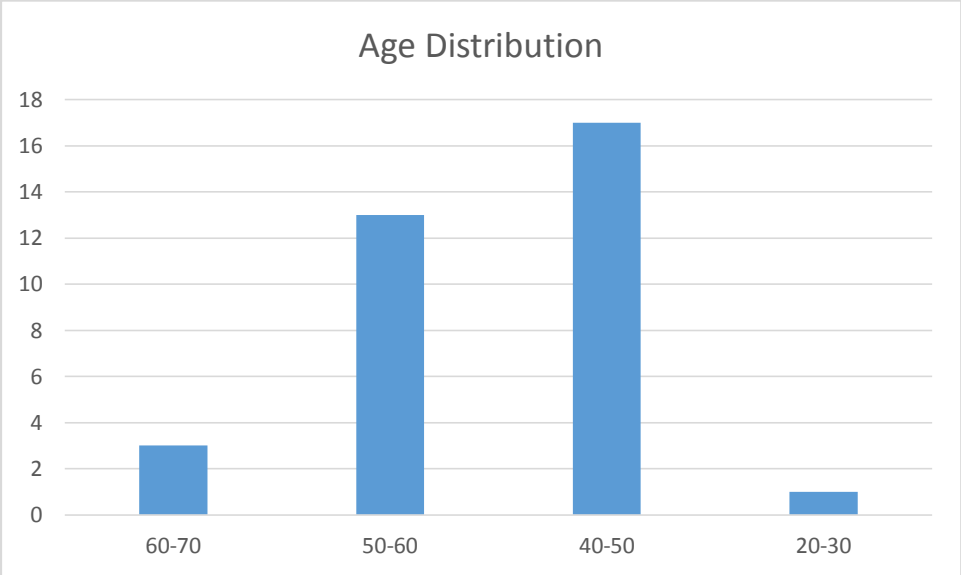
Intervention group													
S.No.	**Name**	**Age**	**Sex**	**Wound bed score Day 1**	**WBS Day 7**	**Wound bed score Day 14**	**Wound swab C&S Day 1**	**Wound swab C&S Day 5**	**Wound swab C&S Day 10**	**RBS day 3**	**RBS day 6**	**RBS day 9**	**RBS day 12**
1	Rajendran	45	M	10	12	14	Peudomonas	Peudomonas	Peudomonas	223	150	201	175
2	Pattammal	54	F	9	11	12	Klebsiella	Klebsiella	No Growth	212	156	186	186
3	Lakshmi	63	F	8	12	14	Staph aureas	No Growth	No Growth	245	187	178	134
4	Venda	55	F	8	11	15	Staph aureas	No Growth	No Growth	167	143	163	187
5	Raju	57	M	7	10	14	Klebsiella	Klebsiella	No Growth	198	198	159	185
6	Raghuraman	58	M	9	12	15	Staph aureas	Staph aureas	Klebsiella	148	150	146	123
7	Tamilselvam	54	M	9	12	13	Staph aureas	No Growth	No Growth	210	163	138	157
8	Mani	52	M	8	11	13	Staph aureas	No Growth	No Growth	230	123	175	186
9	Gajendran	59	M	7	12	14	Klebsiella	Klebsiella	Klebsiella	221	145	186	143
10	Pappu	63	F	8	12	15	Staph aureas	Staph aureas	Klebsiella	240	187	154	167
11	Govindammal	45	F	9	12	14	Staph aureas	Staph aureas	Staph aureus	223	132	163	184
12	Lakshmi 2	49	F	8	11	12	Proteus	Proteus	Proteus	250	156	123	135
13	Victor	48	M	8	9	11	Peudomonas	No Growth	No Growth	230	189	157	169
14	Mary	50	F	9	10	15	Staph aureas	No Growth	No Growth	241	178	168	175
15	Arokiyamary	52	F	9	12	14	Staph aureas	No Growth	No Growth	210	186	201	169
16	Irudhayamary	51	F	7	10	12	Klebsiella	Klebsiella	No Growth	230	207	174	153
17	Raji	53	F	8	11	14	Staph aureas	No Growth	No Growth	231	213	156	183
18	Muniamma	55	F	7	10	13	Proteus	Proteus	No Growth	201	225	183	137

Age Distribution

Statistics

Age

N	Valid	36
	Missing	0
Mean		53.47
Median		53.50
Std. Deviation		7.930
Range		45
Minimum		25
Maximum		69

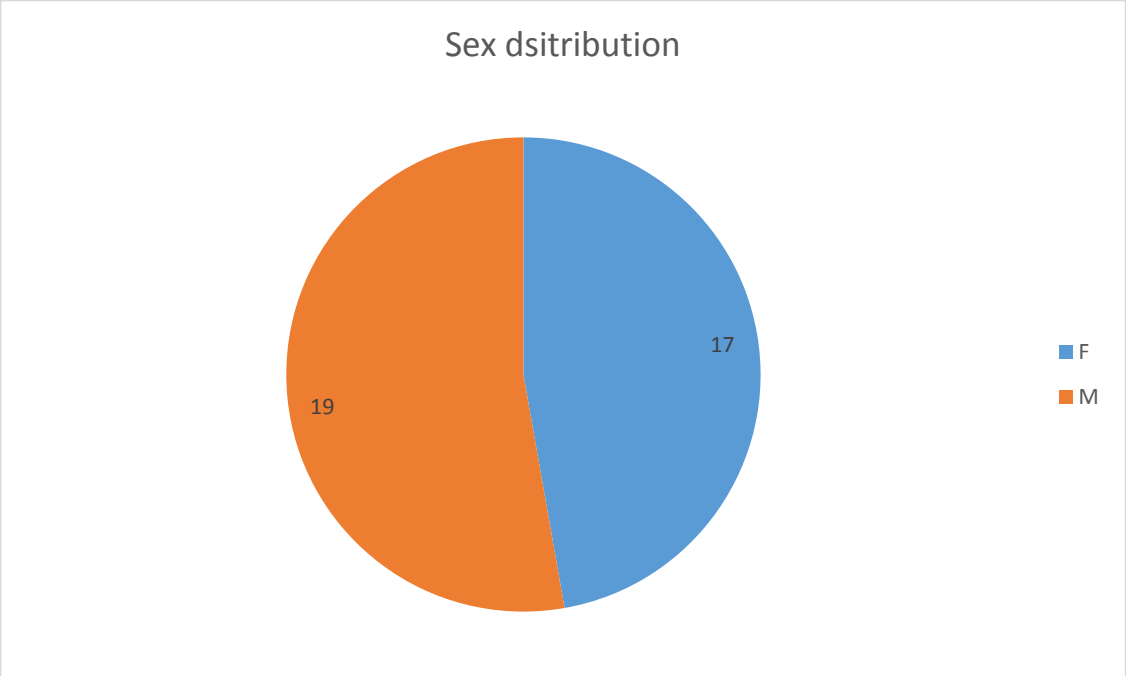


The majority of the subjects were in the 40-60 age group. The youngest was 25 and the oldest patient was 69.

Sex Distribution

Sex

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	F	17	47.2	47.2	47.2
	M	19	52.8	52.8	100.0
	Total	36	100.0	100.0	



The study population had an almost equal distribution of male and female subjects, with 17 females and 19 males.

Wound bed score analysis

The wound bed score at day1, day 7 and day 14 between control and test groups were recorded.

Control group							Intervention group						
S.No.	Name	Age	Sex	Wound bed score			S.No.	Name	Age	Sex	Wound bed score		
				Day 1	Day 7	Day 14					Day 1	Day 7	Day 14
1	Ravi	25	M	8	10	13	1	Rajendran	45	M	10	12	14
2	Ramnath	55	M	7	10	11	2	Pattammal	54	F	9	11	12
3	Eswari	60	F	9	11	13	3	Lakshmi	63	F	8	12	14
4	Loorthumary	48	F	8	9	12	4	Venda	55	F	8	11	15
5	Arokiyamary	56	F	9	11	13	5	Raju	57	M	7	10	14
6	Mariamma	55	F	8	10	12	6	Raghuraman	58	M	9	12	15
7	Muniyamma	57	F	7	9	10	7	Tamilselvam	54	M	9	12	13
8	Vignesh	49	M	8	10	13	8	Mani	52	M	8	11	13
9	Ramesh	62	M	9	11	15	9	Gajendran	59	M	7	12	14
10	Badhushah	65	M	7	10	12	10	Pappu	63	F	8	12	15
11	Afzal	53	M	8	11	13	11	Govindammal	45	F	9	12	14
12	Kannan	52	M	8	10	14	12	Lakshmi 2	49	F	8	11	12
13	Gokulakannan	45	M	9	12	12	13	Victor	48	M	8	9	11
14	Ambiga	46	F	7	10	15	14	Mary	50	F	9	10	15
15	Srinivasan	48	M	9	9	11	15	Arokiyamary	52	F	9	12	14
16	Ramachandran	69	M	8	10	14	16	Irudhayamary	51	F	7	10	12
17	Arumugam	50	M	9	11	13	17	Raji	53	F	8	11	14
18	Gajendran	66	M	8	12	13	18	Muniamma	55	F	7	10	13

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Wound bed score Day 1	Intervetion Group	18	8.22	.878	.207
	Control Group	18	8.11	.758	.179
WBS Day 7	Intervetion Group	18	11.11	.963	.227
	Control Group	18	10.33	.907	.214
Wound bed score Day 14	Intervetion Group	18	13.56	1.199	.283
	Control Group	18	12.72	1.320	.311

There were a total of 18 subjects in either group. The mean wound bed score on day 1 was 8.11 with a standard deviation of 0.758 for the control and 8.22 with standard deviation of 0.878 for the intervention group. The mean wound bed score on day 7 was 10.33 for the control group with a standard deviation of 0.907 and 11.11 with a standard deviation of 0.963 for the intervention group. On day 14 the mean value was 12.72 for the control group with a standard deviation of 1.320 and 13.56 with a standard deviation of 1.199 for the intervention group.

Control group - Mean wound bed score				
Day 1	Day 7	Day 14	Improvement Day 7 over Day 1	Improvement Day 14 over Day 1
8.11	10.33	12.72	2.22	4.61

Intervention group - Mean wound bed score				
Day 1	Day 7	Day 14	Improvement Day 7 over Day 1	Improvement Day 14 over Day 1
8.22	11.11	13.56	2.89	5.34

Improvement of wound bed score			
Day 7 over Day 1		Day 14 over Day 1	
Control	Intervention	Control	Intervention
2.22	2.89	4.61	5.34

The mean wound bed score of control group on day 1 was 8.11 which improved to 10.33 on day 7 and 12.72 in day 14. In the intervention group the mean wound bed score was 8.22 on day one which increased to 11.11 on day 7 and finally to 13.56 on day 14. The Improvement on wound bed score on Day 7 over Day 1 was 2.22 in the control group and 2.89 in the intervention group. The wound bed score on day 14 improved by 4.61 in control group and 5.34 in the intervention group over the day 1 bed score.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Wound bed score Day 1	Equal variances assumed	.636	.431	.406	34	.687	.111	.273	-.445	.667
	Equal variances not assumed			.406	33.294	.687	.111	.273	-.445	.667
WBS Day 7	Equal variances assumed	.086	.771	2.493	34	.018	.778	.312	.144	1.412
	Equal variances not assumed			2.493	33.879	.018	.778	.312	.144	1.412
Wound bed score Day 14	Equal variances assumed	.006	.940	1.983	34	.056	.833	.420	-.021	1.687
	Equal variances not assumed			1.983	33.692	.056	.833	.420	-.021	1.688

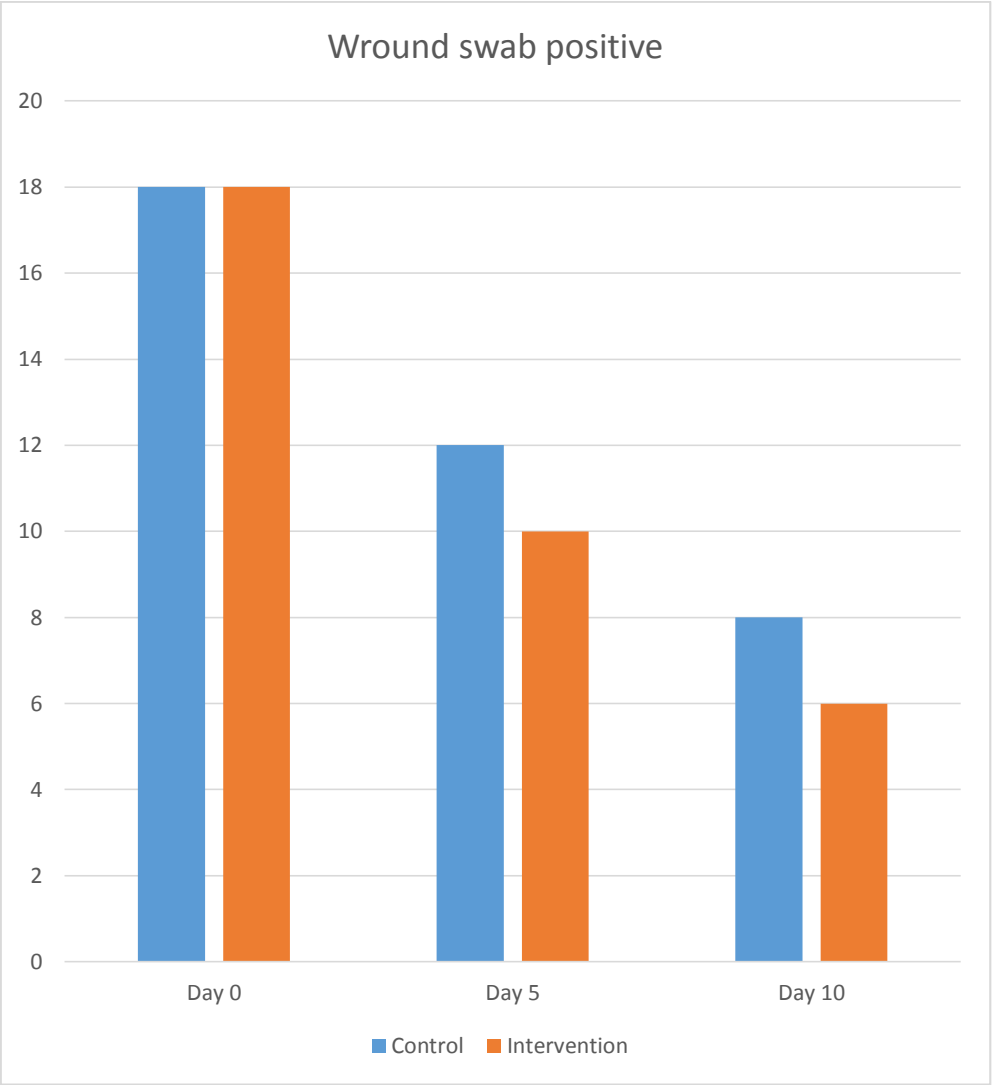
The mean WBS on Day 1 for Intervention group and control group were similar around 8. There were differences in the mean of day 7 (intervention 11.11 vs control 10.33) and in the mean of day 14 (intervention 13.56 vs control 12.72). The difference on WBS on day 7 attained statistical significance (0.018). The difference on WBS on day 14 did not attain statistical significance but was close to significant (0.056, p value 0.05).

Wound swab C&S analysis

The wound swab was sent for analysis on day 1, day 5 and day 10. Those with positive results were grouped as one and with no growth as another.

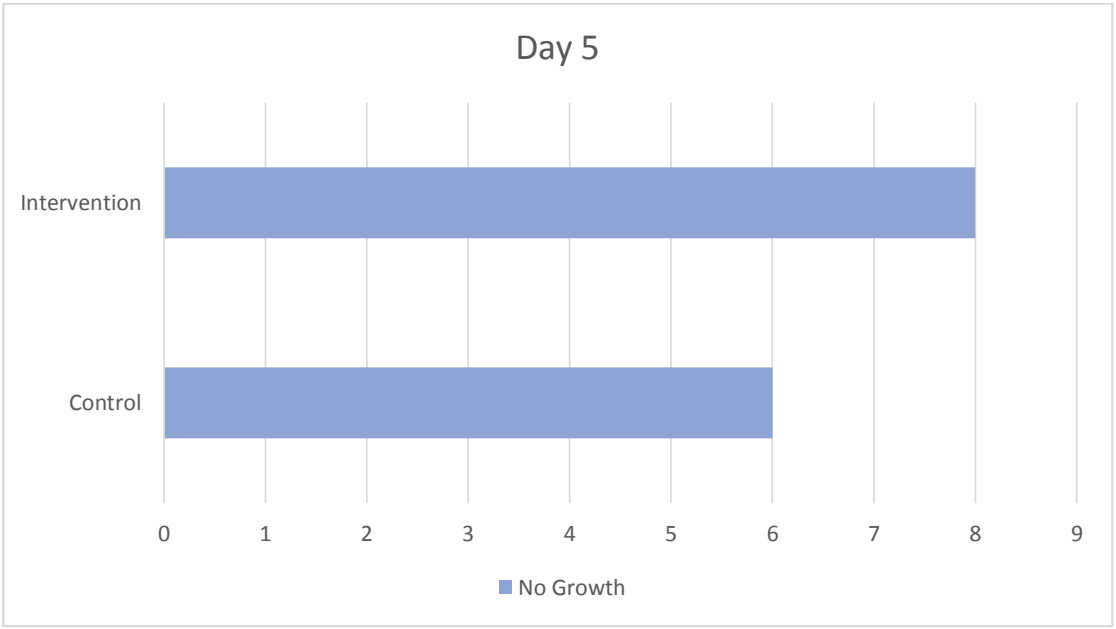
S.No.	Name	Age	Sex	Wound swab C&S Day 1	Wound swab C&S Day 5	Wound swab C&S Day 10	S.No.	Name	Age	Sex	Wound swab C&S Day 1	Wound swab C&S Day 5	Wound swab C&S Day 10
1	Ravi	25	M	Positive	Positive	Negative	1	Rajendran	45	M	Positive	Positive	Positive
2	Ramnath	55	M	Positive	Negative	Negative	2	Pattammal	54	F	Positive	Positive	Negative
3	Eswari	60	F	Positive	Positive	Negative	3	Lakshmi	63	F	Positive	Negative	Negative
4	Loorthumary	48	F	Positive	Negative	Negative	4	Venda	55	F	Positive	Negative	Negative
5	Arokiyamary	56	F	Positive	Positive	Positive	5	Raju	57	M	Positive	Positive	Negative
6	Mariamma	55	F	Positive	Negative	Negative	6	Raghuraman	58	M	Positive	Positive	Positive
7	Muniyamma	57	F	Positive	Positive	Negative	7	Tamilselvam	54	M	Positive	Negative	Negative
8	Vignesh	49	M	Positive	Positive	Positive	8	Mani	52	M	Positive	Negative	Negative
9	Ramesh	62	M	Positive	Negative	Negative	9	Gajendran	59	M	Positive	Positive	Positive
10	Badhushah	65	M	Positive	Negative	Negative	10	Pappu	63	F	Positive	Positive	Positive
11	Afzal	53	M	Positive	Positive	Positive	11	Govindammal	45	F	Positive	Positive	Positive

12	Kannan	52	M	Positive	Positive	Positive	12	Lakshmi 2	49	F	Positive	Positive	Positive
13	Gokulakannan	45	M	Positive	Positive	Positive	13	Victor	48	M	Positive	Negative	Negative
14	Ambiga	46	F	Positive	Positive	Positive	14	Mary	50	F	Positive	Negative	Negative
15	Srinivasan	48	M	Positive	Negative	Negative	15	Arokiyamary	52	F	Positive	Negative	Negative
16	Ramachandran	69	M	Positive	Positive	Negative	16	Irudhayamary	51	F	Positive	Positive	Negative
17	Arumugam	50	M	Positive	Positive	Positive	17	Raji	53	F	Positive	Negative	Negative
18	Gajendran	66	M	Positive	Positive	Positive	18	Muniamma	55	F	Positive	Positive	Negative



The number of patients testing positive for wound swab progressively decreased over the course of treatment.

Day 5



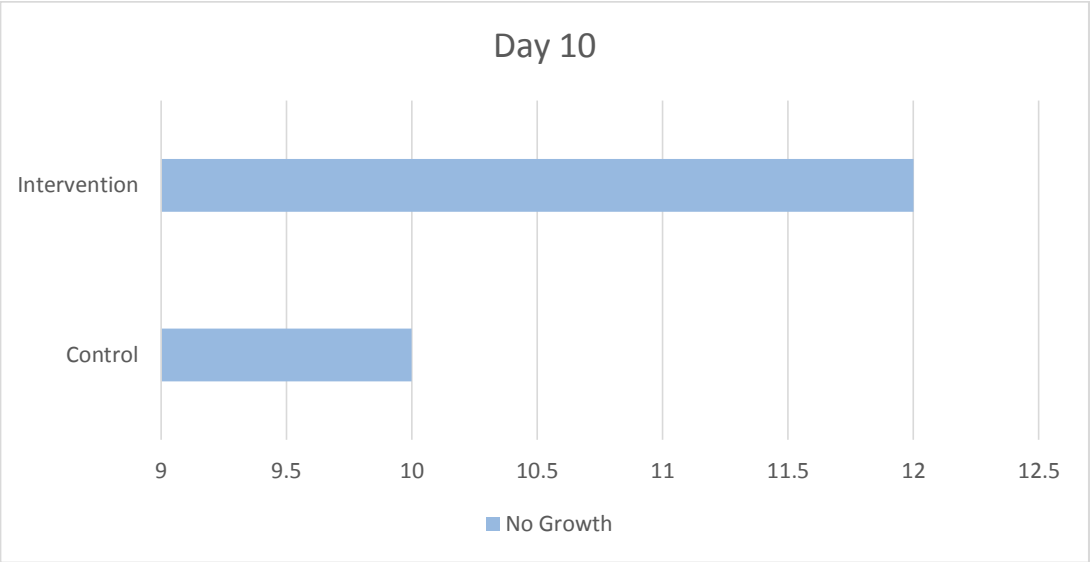
Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.468[b]	1	.494		
Continuity Correction[a]	.117	1	.732		
Likelihood Ratio	.469	1	.494		
Fisher's Exact Test				.733	.367
Linear-by-Linear Association	.455	1	.500		
N of Valid Cases	36				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.00.

Day 10



Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.468[b]	1	.494		
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Linear-by-Linear Association	.455	1	.500		
N of Valid Cases	36				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.00.

We used a chi square analysis to identify any statistical significance in the wound swab cultures. But no significant associated could be found.

Discussion

In our study, a total of 40 patients were enrolled. However 4 of them (2 from each group) could not complete the study as they had to leave the hospital for personal problems. Patients were debrided on the same day and insulin started for everyone with diabetes mellitus. Wound swab was taken on first day. The wound size was measured using tracing method. They were assessed for peripheral artery disease using handheld Doppler, and screened for peripheral neuropathy. These patients were included in the study after checking the inclusion and exclusion criteria and obtaining consent. They were allotted into their group based on sequentially numbered, opaque sealed envelopes.

There were 18 subjects in either group. Majority of them were in 40-60 age group and almost equally distributed between male and female. At the start of the study post debridement, either group had similar mean wound bed score, 8.11 in the control group and 8.22 in the intervention group. The difference was just 0.11. On day 7 however, the difference in the mean was 0.78. Independent samples test (t test for equality of means) showed a significant difference (p value 0.018). On day 14 the difference in mean was 0.84 and was not significant.

The wound swab culture of the wounds were studied on day 0 day 5 and day 10. The number of wounds with a positive status came down as the course progressed in either group. At the end of day 5, 8 subjects in the intervention had negative wound swab cultures while in the control group only 6 had negative wound swab cultures. On day 10, 12 subjects in the intervention group had

negative wound cultures while the control group had 10. Chi square analysis was done and no significance could be attributed to the difference.

Conclusion

1. Probiotics can be safely utilized in therapy of infected diabetic wounds
2. They do hasten the wound healing process as evidenced by the significant difference in the day 7 wound bed score
3. More studies are needed in this field to give better evidence for the support of probiotic use

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Proforma

Name	Age/Sex	IP Number
Intervention/Control group	Date of admission	Date of discharge
Surface are of wound	Ankle brachial pressure index	Vibratory sensation testing
Wound bed score Day 0	Day 7	Day 14
Wound swab C&S Day 0	Day 5	Day 10

RBS Day 3	Day 6	Day 9	Day 12

Master chart

Control													
S.No.	Name	Age	Sex	Wound bed score Day 1	WBS Day 7	Wound bed score Day 14	Wound swab C&S Day 1	Wound swab C&S Day 5	Wound swab C&S Day 10	RBS day 3	RBS day 6	RBS day 9	RBS day 12
1	Ravi	25	M	8	10	13	Staph aureus	Staph aureus	No Growth	224	198	145	156
2	Ramnath	55	M	7	10	11	Pseudomonas	No Growth	No Growth	230	165	187	153
3	Eswari	60	F	9	11	13	Klebsiella	Klebsiella	No Growth	201	123	136	153
4	Loorthumary	48	F	8	9	12	Staph aureus	No Growth	No Growth	180	156	111	164
5	Arokiyamary	56	F	9	11	13	Staph aureus	Staph aureus	Staph aureus	175	178	123	178
6	Mariamma	55	F	8	10	12	Pseudomonas	No Growth	No Growth	146	196	187	193
7	Muniyamma	57	F	7	9	10	Klebsiella	Klebsiella	No growth	189	158	154	165
8	Vignesh	49	M	8	10	13	Staph aureus	Staph aureus	Staph aureus	153	200	132	128
9	Ramesh	62	M	9	11	15	Staph aureus	No Growth	No Growth	169	153	169	197
10	Badhushah	65	M	7	10	12	Staph aureus	No Growth	No Growth	201	168	180	153
11	Afzal	53	M	8	11	13	Pseudomonas	Pseudomonas	Pseudomonas	260	175	150	120
12	Kannan	52	M	8	10	14	Pseudomonas	Pseudomonas	Pseudomonas	302	146	130	102
13	Gokulakannan	45	M	9	12	12	Staph aureus	Staph aureus	Staph aureus	205	189	146	154
14	Ambiga	46	F	7	10	15	Klebsiella	Klebsiella	Klebsiella	260	150	159	163
15	Srinivasan	48	M	9	9	11	Pseudomonas	No growth	No growth	214	136	168	187
16	Ramachandran	69	M	8	10	14	Staph aureus	Staph aureus	No Growth	198	175	145	193
17	Arumugam	50	M	9	11	13	Pseudomonas	Pseudomonas	Pseudomonas	186	142	129	154
18	Gajendran	66	M	8	12	13	Staph aureus	Staph aureus	Staph aureus	173	126	136	130

Intervention group													
S.No.	Name	Age	Sex	Wound bed score Day 1	WBS Day 7	Wound bed score Day 14	Wound swab C&S Day 1	Wound swab C&S Day 5	Wound swab C&S Day 10	RBS day 3	RBS day 6	RBS day 9	RBS day 12
1	Rajendran	45	M	10	12	14	Peudomonas	Peudomonas	Peudomonas	223	150	201	175
2	Pattammal	54	F	9	11	12	Klebsiella	Klebsiella	No Growth	212	156	186	186
3	Lakshmi	63	F	8	12	14	Staph aureas	No Growth	No Growth	245	187	178	134
4	Venda	55	F	8	11	15	Staph aureas	No Growth	No Growth	167	143	163	187
5	Raju	57	M	7	10	14	Klebsiella	Klebsiella	No Growth	198	198	159	185
6	Raghuraman	58	M	9	12	15	Staph aureas	Staph aureas	Klebsiella	148	150	146	123
7	Tamilselvam	54	M	9	12	13	Staph aureas	No Growth	No Growth	210	163	138	157
8	Mani	52	M	8	11	13	Staph aureas	No Growth	No Growth	230	123	175	186
9	Gajendran	59	M	7	12	14	Klebsiella	Klebsiella	Klebsiella	221	145	186	143
10	Pappu	63	F	8	12	15	Staph aureas	Staph aureas	Klebsiella	240	187	154	167
11	Govindammal	45	F	9	12	14	Staph aureas	Staph aureas	Staph aureus	223	132	163	184
12	Lakshmi 2	49	F	8	11	12	Proteus	Proteus	Proteus	250	156	123	135
13	Victor	48	M	8	9	11	Peudomonas	No Growth	No Growth	230	189	157	169
14	Mary	50	F	9	10	15	Staph aureas	No Growth	No Growth	241	178	168	175
15	Arokiyamary	52	F	9	12	14	Staph aureas	No Growth	No Growth	210	186	201	169
16	Irudhayamary	51	F	7	10	12	Klebsiella	Klebsiella	No Growth	230	207	174	153
17	Raji	53	F	8	11	14	Staph aureas	No Growth	No Growth	231	213	156	183
18	Muniamma	55	F	7	10	13	Proteus	Proteus	No Growth	201	225	183	137